in the occurrence of chromosomal gaps and breaks in the bone marrow cells. The highest dose (10 mg/kg) induced a 325% increase in the incidence of gaps and breaks in chromosomes of bone marrow cells. The same dose of 3-quinuclidinyl benzilate had resulted in only a 107% increase in the incidence of chromosomal gaps and breaks; thus, diethylaminoethyl benzilate, despite its lower anticholinergic activity, was more toxic cytogenetically than 3-quinuclidinyl benzilate.

EFFECTS ON MAN

Davies (220) summarized the effects of diethylaminoethyl benzilate at various doses on human beings. Moderate doses--1-4 mg--characteristically produced a sense of divorce between outer reality and emotional reactions. Larger doses--4-8 mg--produced a more distinct detachment: muscles were felt to be relaxed and limbs were sensed as awkward, enlarged appendages with blunted sensations. The ability to concentrate was felt to be diminished, and thought processes were slowed. Mental vacuity was common, and reaction to external stimuli was slow. Continuity in thinking was lost, so that people who had taken doses in this range frequently said something like: "I have forgotten what I wanted to say." Complaints of mild palpitation and of dryness of the mouth might be made. The most common complaint among 110 patients treated with diethylaminoethyl benzilate was of a loss of ability to concentrate and a feeling of detachment from the people and objects in the immediate environment. All doses were given by mouth, the highest dose administered to this group of people being 16 mg/d for several months. No delayed effects or effects that persisted after benzilate administration was stopped were reported.

Raymond and Lucas (221) studied the effects of diethylaminoethyl benzilate on a group of 43 outpatients and 10 normal subjects. Patients given daily oral doses of the drug of 1 mg three times a day (t.i.d.) complained of no side effects. About half the patients who took 2 mg t.i.d. experienced side effects, particularly a sensation of limb heaviness. Most patients given 3 mg t.i.d. complained of side effects, including a feeling of heaviness or rubberiness of the legs, ataxia and clumsiness, difficulty in reading small print, poor ability to maintain continuity of thought or attention, giddiness, diarrhea, anxiety, and drowsiness. The 10 normal subjects were given single subcutaneous injections of 5 mg and then had their EEGs recorded. Five had markedly reduced alpha rhythms in their EEGs. Complaints made by these subjects after they had received the drug, in order of decreasing frequency, were weakness; headache, lightheadedness, and a feeling of heaviness of the limbs; drowsiness; and increased complexity and coloration of photic pattern and facial numbness. Complaints expressed only by some of the 10 subjects were dry mouth, difficulty in expressing thoughts, fear approaching panic, sensation that the floor was distant or seemed to slope, huskiness of voice, heaviness of the shoes, slight nausea, and sweating.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

Kinross-Wright and Moyer (222) reported the results of giving diethylaminoethyl benzilate to 42 patients. They agreed with others that daily doses of 1 mg produced no effects that could be distinguished from those of a placebo. Side effects were infrequent with daily doses of less than 4 mg. With daily doses of 4-8 mg, there were complaints of dryness of the mouth, pupillary dilatation, slight palpitations, and difficulty in concentration. Daily doses of more than 8 mg resulted in such complaints by half the patients, with the addition in some of nausea, anorexia, and constipation. Patients given daily doses of 12 mg or more were affected also by feelings of heaviness of the limbs and of mental confusion, emotional lability and feelings of unreality, drowsiness, dizziness, and ataxia. One patient developed a maculopapular rash.

Vojtechovsky et al. (223) summarized their trials of administering doses of up to 75 mg of diethylaminoethyl benzilate to 17 healthy volunteers. The state of toxic psychosis--resembling a combination of psychoses due to atropine intoxication, to chronic alcoholic damage of the brain stem (Korsakov's syndrome), and to chronic alcoholic delirium--lasted for 4-6 h. They found no somatic complications after doses of up to 75 mg. These investigators suggested that the combination of anticholinergic activity and ability to inhibit monoamine oxidase (resulting in the demonstrated interference with the metabolism of serotonin and possibly of catecholamine transmitters as well) explains the phenomena of intoxication by this benzilate.

Rickels et al. (224) reported the findings in an experiment in which 52 patients were given tablets containing 1 mg of diethylaminoethyl benzilate and were instructed to take five tablets a day, but were allowed to decrease that to three tablets a day if it seemed desirable and, for short times, to two a day. The group of patients studied consisted of approximately equal numbers of psychiatric patients (mildly to moderately depressed neurotic outpatients) and patients seen in general medical practice. A placebo group (45 people) of similar composition received tablets of identical appearance and with the same instructions. The side effects experienced by the subjects were recorded 2 and 4 wk after beginning ingestion of the tablets. The six subjects who had taken a daily average of two or fewer tablets for a 2-wk period were excluded from the final compilations of data. Mean intakes of the two types of tablets were not stated, but would be expected to be about 4.5 tablets a day on the basis of reported intakes of similar tablets containing other drugs involved in the same experiment.

Of the 45 persons who took the placebo for 2 wk, 23 (51%)complained of side effects; 15 (33%) complained of sedation. Of the 52 persons who took the benzilate for 2 wk, 26 (50%) complained of side effects; 15 (29%) complained of sedation. Of the 30 persons who took the placebo for 4 wk, 17 (57%) reported side effects; 11 (37%) complained of sedation. Of the 26 persons who took the benzilate for 4 wk, 10 (38%) mentioned side effects; 4 (15%) complained of sedation. It is apparent that the persons who ingested diethylaminoethyl benzilate had no greater incidence of side effects than those who ingested the placebo.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

Sidell (225) surveyed studies of the effects of 3-quinuclidinyl benzilate on human subjects carried out by Edgewood Arsenal or its contractors between 1960 and 1969. The initial studies were by and on four members of the research staff, who took the compound to become familiar with its effects, so that they could describe to potential volunteers the experiences that they might have as experimental subjects and could devise appropriate means for measuring functional impairment and safeguarding the subjects from physical harm. Sidell was able to identify 314 other subjects who had been given 3-quinuclidinyl benzilate at the Biomedical Laboratory at Edgewood Arsenal or at other sites. In addition, he reported that there had been 24 cases of accidental exposure to 3-quinuclidinyl benzilate among employees of Edgewood Arsenal. Of the 24, 14 had no effects other than mydriasis; four others complained of dry mouth, fatigue or sleepiness, photophobia, and inability to accommodate for near vision; six had difficulty in concentrating on a topic, slowed thought processes, and mild confusion. No long-term effects were reported.

In 1964 and 1969, field tests had been conducted with 3-quinuclidinyl benzilate. As a part of each test, eight volunteers were exposed to monitored aerosolized benzilate and were then assigned model military tasks to perform. A contractor had performed a study of the effect of exposure to this benzilate on the ability of trained pilots to fly airplanes, using the Link flight simulator and other appropriate measures of performance. The procedures used in 23 studies with 3-quinuclidinyl benzilate were outlined by Sidell (225), who also summarized the effects of the compound on man and stated that it is about 20 and 3 times as potent as atropine and scopolamine, respectively, in altering functions of the central nervous system. When equipotent doses were used, the effects of 3-quinuclidinyl benzilate lasted about 6 and 9 times as long as those of atropine and scopolamine, respectively.

Sidell's description of the effects induced by 3-quinuclidinyl benzilate is as follows:

> At low doses, the effects include a dry mouth, decreased gastric mobility, inhibition of sweating, an increase in heart rate, pupillary dilatation and loss of accommodation, mild sedation and mental slowing.
>
> At high doses these effects are intensified. There are marked disturbances of function at all levels of the central nervous system: motor coordination, attentiveness and control of thought and learning processes all decline. Confusion, restlessness, impairment in perception, interpretation, and memory span, poor judgment and deficient insight are all features of this syndrome. True hallucinations are present. If the dose is quite high the subject may become stuporous or even comatose for several hours.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

Freedman (226) reported the findings of a research project designed to determine whether small amounts of 3-quinuclidinyl benzilate modify undesirably the ability of experienced pilots to perform the tasks involved in flying an airplane. The 18 pilots chosen to take part in the study, from a panel of 41 potential subjects, were 25-45 yr old (mean, 38 yr) and had flying times of 200-5,000 h (mean, 1,800 h).

A Link flight simulator and 14 tasks, including the Number Facility Test, were used to assess the competence of the subjects before and at intervals after intramuscular injection of 3-quinuclidinyl benzilate at 1-4 µg/kg. These tasks yielded a total of 35 items that were graded in cycles during each test period. Practically no effects followed doses of 0.5 and 1.0 ug/kg. After doses of 2 µg/kg, there were slight decrements in performance in some of the tasks, a definite increase in heart rate, a decrease in systolic blood pressure, and a slight increase in diastolic blood pressure. The subjects given 4 µg/kg all made lower scores on the performance tests and exhibited definite functional changes, including increased heart rate, dizziness, drowsiness, mydriasis, and difficulty in accommodating the eyes for near vision. Most of the subjects had increased diastolic blood pressure and complained of dryness of the mouth.

The tests associated with the Link flight simulator were the first to demonstrate effects of the 4-µg/kg dose; especially striking changes occurred in vigilance and in judgments of altitude, headings, air speed, and glide paths. These decrements appeared between 1 and 1.5 h after injection. By 3 h, half the subjects could not have completed a mission involving simply taking off and landing an airplane. None could have completed a moderately involved mission. The abilities of the pilots to perform the standard tasks presented to them decreased further up to the end of the observation period, 6 h 40 min after the benzilate had been administered.

In July 1962, it was estimated (227) that exposure of a human population to a Ct product of 3-quinuclidinyl benzilate of 118 mg.min/m^3 would result in incapacitation of 30% of the population, a Ct product of 170 mg.min/m^3 50% of the population, and a Ct product of 347 mg.min/m^3 84% of the population.

Ketchum (228) summarized the experiments with 3-quinuclidinyl benzilate performed between August 1960 and July 1963 with human volunteers. The report included an appendix, prepared by Claude McClure, Jr., on the chemistry and biochemistry of 3-quinuclidinyl benzilate. The outstanding information derived from this appendix is that the brain was the only organ of mice found to contain ^3H 48 h after intraperitoneal injection of [^3H]3-quinuclidinyl benzilate. Perfusion of this benzilate through the livers of rats had indicated that the liver destroyed the compound quickly, 90% of the compound having disappeared from the perfusate within 30 min and less than 0.2% of the compound being found unaltered in the bile. The products were not known. Several hypotheses of biochemical mechanisms of action of 3-quinuclidinyl benzilate were presented, but no firm support for any of them was available.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

The main report concerned 290 exposures of 215 subjects other than staff members to 3-quinuclidinyl benzilate by intravenous injection (11.3%), intramuscular injection (39.3%), ingestion (12.4%), inhalation (21.7%), and application to the skin (14.8%).

Intravenous and intramuscular injections were considered to be equal in effectiveness, although changes occurred more rapidly after intravenous than after intramuscular injection. By ingestion, 3-quinuclidinyl benzilate was about 0.9 times as effective as it was by injection. Inhaled 3-quinuclidinyl benzilate was about 0.6 times as effective as it was by injection. Application to the skin in solution in benzyl alcohol resulted eventually (after 24-36 h) in a group of effects representative of the responses to an injection of about one-seventh the amount.

Estimates of the doses required to be inhaled (expressed as Ct, $mg.min/m^3$) to produce various effects were as follows: mild incapacitation (moderate dilatation of pupils and slight blurring of vision, minimal incoordination, some slowing of thought), 66-124; moderate incapacitation (hallucination, confusion, unorganized hyperactivity, incoherent speech, shortening of memory and attention span), 102-152; and severe incapacitation (stupor or coma, possibly preceded by a period of agitation, followed after 10-15 h by prolonged hallucination and inappropriate behavior), 110-165. The LD_{50} for a man was estimated to be 5.7-6.7 µg/kg.

Repeated daily exposures of volunteers to intramuscularly injected 3-quinuclidinyl benzilate at 1 µg/kg yielded no evidence of cumulative effects, but 2 µg/kg induced clear signs of cumulation. One of 12 volunteers given daily doses of 1-2 µg/kg appeared to develop tolerance.

Repetition of a second administration of 3-quinuclidinyl benzilate 2-3 wk after an initial dose induced a more rapid and more pronounced development of intoxication than the first dose; that suggested a sensitization mechanism in the response to the second dose.

Tetrahydroaminoacridine (THA) had been found to decrease heart rate when tachycardia was present, to increase secretion of saliva, to increase sweating and lower core temperature if it had become high, to reduce muscular rigidity, and to improve, at least temporarily, the mental status of an intoxicated subject. THA seemed not to antagonize sleepiness and might have promoted it. Physostigmine, given orally at 2-6 mg/h, caused nearly total remission of toxic effects within about 8 h after administration of the benzilate. Physostigmine seemed to be a more complete antagonist of the effects of 3-quinuclidinyl benzilate than was THA.

In 1965, the Directorate of Medical Research of the Chemical Research and Development Laboratories, Edgewood Arsenal, issued a booklet (229) on the management of casualties from 3-quinuclidinyl benzilate. The booklet gave the following sequence of effects to be expected in persons who had received large doses: in 1-4 h, tachycardia, dizziness, ataxia, vomiting, dry mouth, blurred vision, confusion, and sedation progressing to stupor; in 4-12 h, inability to respond

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

effectively to the environment or to move about; and in 12-96 h, increasing activity, random unpredictable behavior, and gradual return to normal 48-96 h after exposure. The booklet recommended treatment of casualties with intramuscular injection of 3 mg of physostigmine salicylate, followed by another such dose 40 min later, if necessary. Maintenance by oral doses of 2-5 mg of physostigmine salicylate every 1-2 h was suggested. With improvement in mental status, the frequency and size of the maintenance doses can be reduced gradually. Heart rate was recommended as an indicator of the adequacy of therapy. The pulse should be between 70 and 80 beats/min when good control has been achieved. If the heart rate falls below 70 beats/min, the frequency or magnitude, or both, of the maintenance doses of physostigmine salicylate should be reduced, but the patient should be observed carefully for possible reversion to toxic delirium. Peripheral effects of physostigmine overdoses (profuse sweating, clammy skin, abdominal cramps, vomiting, muscular twitching, tremor, and weakness) may be antagonized by small doses of methylatropinium nitrate or, if that is not available, of atropine itself.

Kitzes and Vancil (230) reported the results of a study to determine the intramuscular dose of 3-quinuclidinyl benzilate that constitutes the minimal effective dose (MED) for a man, here defined as the smallest dose that produces a decrease of at least 25% in performance in the Number Facility Test. The effects on heart rate were also recorded. Thirteen male volunteers were given intramuscular injections of 3-quinuclidinyl benzilate hydrochloride at 2.3 or 2.7 µg/kg. One man given 2.7 µg/kg became confused and developed hallucinations. The most common complaints of the other volunteers were of dry mouth, blurred vision, and drowsiness. Several developed restlessness, an inability to sleep despite drowsiness, and anorexia. Of six men given 2.3 µg/kg, one had a decrease in performance in the Number Facility Test of more than 25%, and three had dilated pupils. This dose induced no tachycardia or hyperthermia. Of seven men given 2.7 µg/kg, five had decreased performance in the Number Facility Test of more than 25%, three had dilated pupils, two had heart rates greater than 100 beats/min, and one had a blood pressure above 140/90. There were no cases of hyperthermia (the report did not state the environmental conditions of the study). The mean heart rate after the larger dose was about 92 beats/min, whereas that after the smaller dose was about 79 beats/min.

Kitzes and Vancil estimated that the MED related to performance in the Number Facility Test was 2.54 µg/kg, with 95% confidence limits of 2.31 and 2.80 µg/kg, and that the MED for inducing changes in somatic functions (vision, heart rate, and blood pressure) was about 2.7 µg/kg.

Crowell (231) described experiments in which 27 male volunteers were given intramuscular L-2-alpha-tropinyl benzilate at 1.9-8.6 µg/kg after a previous unpublished estimate that the intravenous MED was about 3.8 µg/kg. This benzilate was found to have a quicker onset of effects after intramuscular injection and a briefer duration of action than 3-quinuclidinyl benzilate. Every subject who received a dose

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

of 2.8 µg/kg or more experienced dryness of the mouth, blurred vision, and dizziness. Nearly all the subjects given 4.1 µg/kg or more complained of anorexia, nausea, weakness, confusion, hallucinations, and ataxia. Most expressed the illusion that their hands and feet were as red as blood. About one-third suffered headaches, and a few vomited. The intramuscular ED_{50} for inducing a decrement in performance in the Number Facility Test of at least 25% was about 3.1 µg/kg, that for increasing heart rate to at least 100 beats/min was about 5.6 µg/kg, and that for reducing performance in the Number Facility Test by at least 90% was about 5.9 µg/kg. For men given L-2-alpha-tropinyl benzilate at a mean dose of 6.1 µg/kg, the mean duration of severe effects of the compound was 2.5-3.1 h, and the mean time of onset for reduction of performance in the Number Facility Test by at least 90% was about 1 h. At 10 d after intramuscular injection of L-2-alpha-tropinyl benzilate, no evidence of any organic damage attributable to the compound could be found. Crowell's estimate of the incapacitating dose by intramuscular injection was 6.0 ± 0.75 ug/kg.

Kitzes et al. (154) reported the results of 40 experiments in which volunteers with body weights between 59.1 and 96.3 kg were given 3-quinuclidinyl benzilate at 2.0-8.0 µg/kg. These experiments revealed no clear relation between body weight or benzilate dose and performance in the Number Facility Test. As part of the same study, 10 experiments were performed with volunteers with body weights between 65.4 and 87.7 kg who were given L-2-alpha-tropinyl benzilate at 1.9-8.6 µg/kg. With this compound, there seemed to be no clear relation between dose and performance in the Number Facility Test, but there may have been a positive relationship between body weight and performance. When the range of weights of these subjects was divided in half, the upper half of the range included four experiments with men given a mean dose of 5.4 µg/kg and having a mean body weight of 85.3 kg, and the lower half included six experiments with men given a mean dose of 4.3 µg/kg and having a mean body weight of 69.7 kg. Despite the smaller mean dose of the benzilate received by the men in the lower half of the weight range, that half included all the men rated as being the most severely affected in performance in the Number Facility Test. With 3-quinuclidinyl benzilate, the two halves of the weight range contained close to the same proportions of severely affected men: 45.4% in the upper half and 48.3% in the lower half. Here, also, the mean doses for the two halves of the weight range were similar: 4.3 µg/kg for the upper half and 4.7 µg/kg for the lower half.

Ketchum et al. (189) reported the results of field tests in which two groups of four men each were exposed on different days to clouds of an aerosolized solution of 3-quinuclidinyl benzilate in chloroform. After exposure to the cloud, the subjects were rated on their performances in a variety of tests of physiologic and psychologic properties. Baseline performances in these tests had been recorded after 12-20 practice runs before exposure; the five best scores in the practice runs were averaged as the baseline scores. A dry run before the actual experiment incorporated all features of the experiment except the exposure; the scores on the test battery

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

during the dry run did not deviate significantly from the baseline scores. The conclusions from the study were that the ED_{50} for incapacitation by 3-quinuclidinyl benzilate under field conditions is about 60 mg.min/m^3 for a man with a body weight of 75 kg and a minute volume of respiration of 15 L. Intramuscular injections of 2 or 3 mg of physostigmine salicylate were found to reverse the toxic delirium rapidly; repeated injections every 2 or 3 h or oral doses about one-third larger than the intramuscular doses, on the same schedule, restored and maintained acceptable performance in the test battery and in carrying out simulated guard duty of a post perimeter or simulated sentry duty in a foxhole under attack by an aggressor.

Craig et al. (232) reported the results of exposing 24 volunteers to high environmental temperatures at various times after they had been given intramuscular 3-quinuclidinyl benzilate at 3, 4, 5, or 6 µg/kg by intramuscular injection. Two series of exposure to heat were performed. In the first, four subjects were exposed to 41°C for 2 h on each of two days and six subjects were exposed to the same temperature during two 2-h periods on one day; on the third day after the first day of exposure to heat, all 10 subjects were given intramuscular injections of the benzilate during the first of two 2-h exposures to 41°C, separated by 3 h, followed by two similar exposures on the same schedule on the following day and by a single 2-h exposure of six men on the morning of the sixth day of the experiment (four of these men had another 2-h exposure to heat on the afternoon of the same day). In the second series, 14 men were exposed to 41°C for 8 h on day 1, to 52°C for 8 h on day 3, and to 41°C for 8 h on day 7, beginning 4 h after an injection of 3-quinuclidinyl benzilate.

The effects of the benzilate reached their maximum about 6 h after injection. At that time, the heart rate, skin temperature, rectal temperature, and inhibition of sweat secretion were increased in relation to the dose of benzilate administered. The highest rectal temperature was 38.3°C, an increase of less than 1°C above the mean normal temperature. The disturbance of regulation of body temperature by 3-quinuclidinyl benzilate under the condition of recumbent rest used in these experiments should not be hazardous to a healthy person. Physical activity during the exposure to heat might alter this general picture, although a previous study had found that the combination of 3-quinuclidinyl benzilate at 4 µg/kg, exercise, and an environmental temperature of 46°C produced only a slightly greater increase in rectal temperature than the exercise and environmental temperature without the benzilate. The inhibition of sweating by this benzilate regressed at about half the rate at which that induced by atropine regressed.

Klapper et al. (162) reported the results of followup studies on two men who had been given 3-quinuclidinyl benzilate and one who had been given L-2-alpha-tropinylbenzilate earlier. The first two had histories of intermittent hematuria before their service as volunteer subjects; at the times of the followup examinations, they had fewer than 10 red blood cells per high-power field in their urine. One of them reported that he had had a flashback of his experiences under the influence

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

of the benzilate when drinking alcoholic beverages about 3 wk after exposure. There were no remarks about the man who had received the other benzilate.

Klapper et al. (163) examined the records of 24 volunteer subjects who had taken the MMPI test and then had been given intramuscular injections of 3-quinuclidinyl benzilate at 2.1 (six men), 2.4 (11 men), or 4.5 (seven men) µg/kg in an attempt to determine the factors of the MMPI that are best correlated with the response to the benzilate represented by performance in the Number Facility Test. The dose of 2.1 µg/kg produced a mean decrease in performance in the Number Facility Test of 27%, 2.4 µg/kg produced a mean decrease of 26%, and 4.5 µg/kg produced a mean decrease of 74%. The scales of the MMPI that were best correlated with the individual scores in the Number Facility Test were that for positive test-taking attitude for 2.4 µg/kg and that for hysteria for 4.5 µg/kg. There were no significant positive correlations for the lowest dose. There were significant negative correlations with the schizophrenia and the hypochondriasis scales after that dose, but these negative correlations were not reported for either of the larger doses.

Sidell et al. (164) found that S-(2-diisopropylaminoethyl)-ethylmethylphosphonothioate injected intravenously at 1.5-1.7 µg/kg after intramuscular injection of 3-quinuclidinyl benzilate at 6 µg/kg was effective in inducing rapid improvement in the scores of three volunteers in the Number Facility Test. This finding was similar to that reported earlier in subjects exposed to scopolamine.

Sidell (168) reported that four volunteer subjects given intramuscular injections of 3-quinuclidinyl benzilate at 7 ug/kg had their scores in the Number Facility Test improved dramatically by intramuscular injection of 7 or 11 mg of physostigmine salicylate followed by oral administrations of 24 or 12 mg, respectively, of the same drug during total periods of treatment of 43 and 42 h, respectively. Two other subjects given the same dose of the benzilate were treated entirely with oral administration of physostigmine in total doses of 80 and 211 mg during periods of treatment of 37 and 71 h, respectively. The scores in the Number Facility Test of the last two subjects were better maintained than those of the first two.

SUMMARY

3-Quinuclidinyl benzilate, the benzilic acid ester that has been studied the most by Edgewood Arsenal, has effects similar in general to those of the tropic acid esters, but more prolonged. Two other benzilic acid esters have similar actions. In part, the particularly long duration of the central action of 3-quinuclidinyl benzilate may be related to its greater affinity for nervous tissue, and especially for mitochondria within neuronal cells. In agreement with its especially strong adsorption on mitochondria, the subcellular organelles concerned principally with the supply of energy to the cell, this benzilate has been found to reduce the oxygen consumption by nerve cells stimulated in various ways. This

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

and the other two benzilates on which some information was found have some activity as local anesthetic agents and as antihistaminic compounds, in addition to their activity as peripherally and centrally effective antagonists of cholinergic agonists. The only long-term toxicity study of a benzilic acid ester that was found was of the ester with diethylaminoethanol; no significant pathemas were found in rats during this lifetime feeding study, but the lifetimes of the rats fed the benzilate may have been shortened somewhat. Feeding of the ester with quaternized 3-quinuclidinol to rats for a year and gavaging dogs with this compound 5 d/wk for a year produced no significant pathemas.

Both cholinergic and anticholinesterase compounds antagonized the toxic actions of the benzilates. Although 3-quinuclidinyl benzilate has been found to have weak mutagenic activity for yeast cells in culture and to produce gaps and breaks in chromosomes of bone marrow cells, it has not been proved to produce heritable changes in mammals. Diethylaminoethyl benzilate was more toxic cytogenetically than 3-quinuclidinyl benzilate, despite the lower anticholinergic activity of the former compound.

ESTERS OF PHENYLCYCLOPENTYLGLYCOLIC ACID

This group of anticholinergic esters contains five compounds, the subject acid being esterified with the following alchohols: tropine, 3-quinuclidinol, 2-methyl-3-quinuclidinol, N-methyl-4-piperidinol, and a mixture of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol. Of 20 reports on these compounds by personnel of Edgewood Arsenal and its contractors, 10 are on the ester with N-methyl-4-piperidinol, four on the ester with the mixture of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol, and two each on the other three esters. The literature on the ester with N-methyl-4-piperidinol is entirely from Edgewood Arsenal, whereas more has been written about the ester with N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol (Ditran) by investigators other than those at Edgewood Arsenal than by the ones at that site. Most of the information about the other esters in this group originated at Edgewood Arsenal. The following discussion concentrates on the two most popular esters in this group.

The information on the lethal activities of the members of this group of esters is sparse. Even when reports dealing with lethality according to their titles were furnished by the Board on Toxicology and Environmental Health Hazards, the texts provided were brief and uninformative. An example is a report (233) entitled A Review of the Pharmacology and Toxicology of CAR 301,060. This compound is the ester of phenylcyclopentylglycolic acid and 2-methyl-3-quinuclidinol. The entire text reads:

> CAR 301,060, a BZ-like glycolate, is, on the basis of extensive pharmacological evaluation, a potent anticholinergic compound that displays marked behavioral

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

and CNS effects. The compound, in most instances, is equal to or more potent than BZ, and has similar temporal parameters. It is exceptionally unique in regard to profound potency in the ACRBL-HAZLETON visual discrimination test. Extensive acute and subacute toxicological evaluation failed to reveal any extremely profound toxic or pathological effect in any major organ system at the doses tested. CAR 301,060 has a very substantial safety margin in all species tested.

Such pap is a waste of paper and time.

Other reports of that ilk (234-236) give a little useful information. The first of these (234) stated that repeated daily intravenous doses of the phenylcyclopentylglycolic acid ester of N-methyl-4-piperidinol produced mydriasis in monkeys, that doses of 0.1 mg/kg or more resulted in above-normal ratios of adrenal:body and testes:body weights, and that the highest dose administered, 1.0 mg/kg, resulted in unusually small livers. None of the organs mentioned was considered to have been harmed histologically. The second report (235) concerned the effects of the same compound in the dog. The lowest daily intravenous dose, 0.01 mg/kg, induced mydriasis; the two higher doses, 0.1 and 1.0 mg/kg, resulted in ataxia, decreased activity, and dryness of the mouth, in additon to mydriasis. They also induced significant effects on the performance of the dogs in a multiple-stimulus conditioned-avoidance test. The highest dose induced a statistically significant decrease in body weight. At necropsy, no significant effects that could be attributed to the agent were found. The third report in this group (236) described the effects of the phenylcyclopentylglycolic acid ester of 3-quinuclidinol on dogs. Repeated daily intravenous doses of 0.1 mg/kg produced maximal mydriasis that persisted throughout the study, decreased activity and ataxia, decreased concentration of the urine, and significant changes in the weights of the gonads and kidneys. The lower dose, 0.01 mg/kg, was not reported to have produced any effects other than mydriasis and decreased activity. The dogs that had been trained in the multiple-stimulus conditioned-avoidance test displayed a rapid development of tolerance to a daily dose of 0.1 mg/kg.

N-METHYL-4-PIPERIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (EA 3443)

N-Methyl-4-piperidinyl-(phenylcyclopentyl)-glycolate, also designated EA 3443, was found (193) to be effective both prophylactically and therapeutically in reducing the adsorption of 3-quinuclidinyl benzilate by the cat's motor cortex, caudate nucleus, and sensory cortex after intravenous injections of the benzilate and the glycolate. When given after the benzilate, EA 3443 reduced the retention of that compound by the medial and lateral geniculates. When it was injected first, EA 3443 reduced the adsorption of 3-quinuclidinyl benzilate by the hypothalamus and thalamus, in addition to the three parts of the brain first mentioned. Prophylactically administered EA 3443 decreased the adsorption of the benzilate by the

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

hypophysis, liver, lungs, skeletal muscles, spleen, and sciatic nerve. When it was administered to cats after the benzilate, it reduced retention of the benzilate by skeletal muscles, lungs, sciatic nerve, pancreas, liver, spleen, and heart. When added to the fluids surrounding a segment of isolated guinea pig ileum, EA 3443 reduced almost to zero the response to acetylcholine. This glycolate had about the same activity in this regard as 3-quinuclidinyl benzilate, but the anticholinergic effect of the glycolate was the one more readily reversed by tetrahydroaminoacridine.

Kitzes and Ketchum (237) reported giving 39 volunteers intramuscular EA 3443 at 1.0-2.7 µg/kg. These doses induced no hallucinations or abnormal neurologic signs. Dry mouth, blurred vision, and drowsiness were the most common complaints. Mydriasis occurred to a mild degree. There was no consistent dose-response relationship for the heart rate, but that rate may have been decreased, rather than increased. The minimal effective dose for reducing performance in the Number Facility Test of half the subjects by at least 25% (MED_{50}) in this study was 1.21 µg/kg, with 95% confidence limits of 1.00 and 1.48 µg/kg. The time for onset of these minimal effects was about 8 h, and the duration of effects was about 4 h.

Kitzes et al. (154) found that 80% of a group with a mean body weight of 87.2 kg who had been given EA 3443 at a mean dose of 2.2 µg/kg suffered degradation of their performances in the Number Facility Test. Only 46.7% of a group with a mean body weight of 71.6 kg who had received a mean dose of 2.4 µg/kg suffered degradation of their performances in the same test. In the case of this compound, therefore, body size may have conditioned the response.

Hart and Balter (238) exposed an unstated number of volunteers to EA 3443 by application to an unidentified area of the skin at 60-120 µg/kg (mean, 68.5 µg/kg). The volunteers were tested with a modification of the Army General Classification Test before and at 7 wk after EA 3443 exposure. During the exposure, the Number Facility Test was used to determine whether the subjects experienced any degradation of their mental abilities. The criterion for an MED (two of five consecutive scores below 75% of the baseline score) was met by 71% of the exposed volunteers. A control group of 15 volunteers were tested and retested 20 d later without any known intervening exposure to a psychotoxic substance. The mean score of the control group at the second testing was 3.2% below that at the first testing. The second testing of the exposed group yielded a mean score for that group that was 2.4% above that at the first testing. Neither of these differences is significant.

Lavallee (50) reported that the minimal intravenous dose of EA 3443 that decreased performance by at least 25% in the Number Facility Test by half the members of a group of volunteers was 1.2 µg/kg. The corresponding incapacitating dose, the ID_{50}, was 3.1 µg/kg. That for 3-quinuclidinyl benzilate was 6.6 µg/kg. When EA 3443 was injected intravenously into dogs trained in a multiple-stimulus conditioned-avoidance test, a dose of 100 µg/kg decreased the

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

number of correct responses to visual stimuli by about 28.2%. This decrement had been surpassed by that induced by 3-quinuclidinyl benzilate at 32 µg/kg. In monkeys trained in a visual-discrimination avoidance task, intravenous EA 3443 at up to 56 µg/kg had no significant effects on performance of the task. The same doses of 3-quinuclidinyl benzilate also had no significant effects on the responses of monkeys in this test. Lavallee concluded that the screening tests with dogs and monkeys were not satisfactory for predicting the relative potencies in man of the anticholinergic compounds tested.

Klapper et al. (163) found that the scale of the MMPI that was the best correlated with the degradation of performance of volunteers in the Number Facility Test after intramuscular doses of EA 3443 (six volunteers, 1.8 µg/kg; five volunteers, 2.4 µg/kg) was that for paranoia. The lowest negative correlation was that between the social introversion scale and the degradation of performance in the Number Facility Test. These statements were true for both doses of EA 3443; that is different from the situations with some of the other compounds studied in this way. With both atropine and 3-quinuclidinyl benzilate, the larger dose produced a degree of degradation of performance in the Number Facility Test that was best correlated with the score in a different scale of the MMPI from the one that yielded the best correlation with the lower dose of the same agent.

Armstrong et al. (239) used a test that required water-deprived rats to press four levers in a preset sequence to obtain water. After administration of EA 3443 by an unstated route, the ED_{50} for a minimal mydriatic effect was 37 µg/kg; that for reduction of the number of drops of water obtained within a given period was 53.2 µg/kg.

DITRAN (CS 4297)

In 1958, Abood et al. (240) reported initial studies of a group of esters of alkylated derivatives of piperidine, including a material that was thought to be N-ethyl-3-piperidyl-(phenylcyclopentyl)-glycolate. This substance was given the trivial name Ditran; it is known now to be a mixture of two compounds: the phenylcyclopentyl glycolates of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol in the proportion 3:7. This substance was not as centrally active (producing hyperactivity in rats) as the methyl homologue, but was less lethal and more potent as a peripheral anticholinergic. Ditran was administered to more than 40 human volunteers by Ostfeld et al. (241) and was found to be actively hallucinogenic. Esters of 3-piperidyl alcohols were found to be much more hallucinogenic than corresponding esters of 4-piperidyl alcohols.

Abood et al. (242) compared 14 related esters of substituted piperidyl alcohols. They found that the phenylcyclopentyl and phenylcyclohexyl glycolates were more actively hallucinogenic in human subjects than diphenyl, phenylpropyl, or phenyl 2-thienyl glycolates. Esters of quaternized piperidinium alcohols were not hallucinogenic. A

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

hydroxyl group on the carbon atom alpha to the carboxyl group of the acid was found to be essential for hallucinogenic activity. Ditran was found to have an intravenous LD_{50} for the rat of 19 mg/kg and one for the mouse of 44 mg/kg. The latter value may have been erroneous, inasmuch as Usdin and Efron (243) later stated that the intravenous LD_{50} for the mouse was 10 mg/kg. However, Usdin and Efron may have confused the dose given to human subjects by Abood et al., which was 10 mg/man, with the figure for the LD_{50} for the mouse after intravenous injection. The value reported by Abood et al. by this route accords better with the LD_{50} after intraperitoneal injection in the compilation of Usdin and Efron than that stated by the latter authors. Usdin and Efron gave also intravenous LD_{50}s for the guinea pig and the rabbit of 45 and 12 mg/kg, respectively. Intraperitoneal injections of Ditran into mice and rats yielded LD_{50} estimates of 60 and 25 mg/kg, respectively. Little correlation between peripheral anticholinergic activity and psychotogenic potency was found by Abood et al. They reported that intraperitoneal injection of physostigmine at 0.5 mg/kg into rats 5 min before injection by the same route of Ditran at 25 mg/kg prevented mydriasis and lessened tachycardia and hyperemia of the tail and limbs, but had no visible effect on hyperactivity or on weakness and poor coordination of muscles.

Gershon and Olariu (244) assessed the ability of Tacrine to antagonize the actions of Ditran on human subjects and compared the effects of Ditran with those of mescaline, Sernyl, and LSD. The most common complaints of subjects given doses of Ditran by intramuscular injection or by mouth, or by both routes, were of hallucination, confusion, and impaired contact. Gershon and Olariu found that Tacrine was able to antagonize both the peripheral and CNS effects of Ditran in man, but was incapable of preventing those due to Sernyl. Succinate, which effectively reverses both the peripheral and central effects of Sernyl, had no activity against intoxication with Ditran. The authors gave the following typical sequence of signs and symptoms after intramuscular injection of 10–15 mg of Ditran: in 20–30 min, autonomic reactions (dryness of the mouth, slight tachycardia, flushed face, and muscular relaxation); in 45–60 min, central effects (some confusion, speech difficulty, decreased ability to concentrate, slight disorientation, occasional vertigo, and hallucinations); in 2–4 h, maximal central effects (hyperreflexia and ataxia); and in 5–8 h, abatement of symptoms and signs.

Biel et al. (245) reviewed the effects produced by a large group of anticholinergic compounds in an attempt to develop guidelines for the development of new psychotropic drugs and reached the following general conclusions: only the piperidyl and pyrrolidyl glycolates that were potent anticholinergic compounds exerted profound effects on CNS functions. However, peripheral anticholinergic potency did not endow a compound automatically with brain activity. Over 95% of a dose of Ditran is excreted in the urine within 2 h after its administration. The largest brain concentrations of several of these substances were in the hypothalamus and caudate nucleus,

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

areas related intimately with emotion and mood. Psychotropic anticholinergic compounds had little effect on several enzyme systems. Ditran is a clinically effective antidepressant agent. An inhibitor of cholinesterases, tetrahydroaminoacridine, is capable of ameliorating the psychotic episode induced by anticholinergic psychotogens. Piperidine, trifluoperazine, and several other tranquilizers were effective in overcoming hyperactivity induced by anticholinergic compounds in rats.

Bell et al. (85) used dogs to study the psychotropic activity of Ditran and its antagonism. After developing a rating scale including six indexes of autonomically evoked activity, six of motor activity, and eight of central nervous system activity, they were able to present a graph demonstrating that the time courses of each of the major types of effect induced by intravenous Ditran at 0.5 mg/kg had a somewhat individual time course. Mydriasis, tachycardia, and decreased secretion of saliva were apparent within 3 min after injection. Between 3 and 6 min after injection, the pupil response to light became sluggish, and alertness and responsiveness to commands began to decrease. Within 12-15 min after injection, incoordination and ataxia had reached a plateau, and the pupil response to light usually had disappeared. Obstinate progression and disturbed behavior (whining or barking, pawing at the floor, and apparently chasing imaginary objects) became evident at 30-40 min after injection. By an hour after the injection, most of the dogs had collapsed. Prostration, from which the dogs could be roused temporarily by noise or handling, lasted for 15-120 min. The integrated total effect (obtained by summation of scores on the various parts of the rating scale) reached an approximate plateau at about 0.5 h after injection and remained there till about 2.5 h after injection. Approximately complete recovery from the effects of this dose on dogs did not occur until about 5 h after injection. Mydriasis was the last sign of effect by Ditran to disappear, often being evident for up to 8 h after injection.

Bell et al. (85) found that doses of physostigmine or neostigmine injected intravenously at 20 µg/kg 30 min after Ditran antagonized the effects on autonomic functions, but did not alter the central actions. Arecoline (150 µg/kg) restored salivation and heart rate to normal, but had no other detectable antagonistic actions when injected intravenously 30 min after Ditran. Tacrine at 1 mg/kg, injected intravenously 30 min after Ditran, abolished all effects of Ditran except mydriasis and decreased secretion of saliva. About 40% of the dogs so treated had relapses about 10 min after the dose of Tacrine; repetition of the dose of Tacrine induced complete and permanent recovery, except for persistent mydriasis. Some derivatives of glycolic acid (phenanthrylglycolic acids), p-phenylmandelic acid, and benzilic acid injected 30 or 60 min before Ditran shortened by up to 55% the duration of the intoxication due to Ditran, but did not alter the initial severity of its effects. These organic acids exerted less antagonism to intoxication with Ditran when they were

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

administered therapeutically than when they were used prophylactically.

Bell and Gershon (86) reported that yohimbine antagonized the intoxicating actions of Ditran, but differed from Tacrine in this sort of activity, in that its antagonistic activity was less time-dependent than that of Tacrine. The latter had only slight antagonistic activity early in the intoxication and had greater activity when it was administered later after the Ditran, whereas yohimbine had more nearly equal antagonistic activities at various times after the dose of Ditran.

Brown et al. (246) gave 48 dogs intravenous Ditran at 0.5 mg/kg and 30 min later injections of saline, chlorpromazine, imipramine, or two unidentified substances, IN 1060 and W2045. IN 1060 is known also as cyprolidol, an antidepressant, but an equivalent for W2045 could not be found. Chlorpromazine and imipramine deepened the intoxication induced by Ditran, whereas IN 1060 and especially W2045 lightened the intoxication. When chlorpromazine and the two other compounds were fed to dogs daily for a month before the animals were given Ditran, chlorpromazine accentuated the psychotogenic action of Ditran, whereas IN 1060 and W2045 both attenuated the psychotogenic action of Ditran. After the month-long dosing with the last two antagonists, IN 1060 had a much greater effect on the central actions of Ditran than W2045, whereas the reverse had been true after single therapeutic administrations of the two drugs. For both compounds with both schedules of administration, the effects on the central actions of Ditran were greater than those on the motor and autonomic actions.

Banshchikov and Stoliarov (216) compared the effects of three of Biel's compounds--JB-318, JB-329 (Ditran), and JB-336--among themselves and with those of benactyzine and atropine. They also discussed the use of these compounds in the practice of medicine. Ditran had a much less prolonged action than JB-318, which was described as causing loquacity and hyperactivity with an elevated mood that persisted for days, weeks, or even months. In the few people who had been persuaded to take repeat doses of this compound, resistance to the hallucinogenic action developed, but the mydriatic effect persisted. The effects of all three of Biel's compounds were qualitatively similar and resembled those induced by atropine and benactyzine. Daily doses of Ditran were stated to lead to development of resistance to its actions: after 3 wk of daily intake, a dose of 20 mg/kg no longer resulted in mental disturbance. The posthallucinatory euphoriant action of Ditran has been applied to the treatment of patients in depressed states of mind, apparently with some success. Ditran has also been used in treating patients with neuroses and psychoses.

Albanus (42) reported that subcutaneous injection of Ditran at 0.5 mg/kg into 11 dogs resulted in the development of ataxia after a mean of 22 min, of obstinate progression after a mean of 31 min, and of complete loss of contact with the environment after a mean of 45 min. Obstinate progression disappeared about 230 min after the injection of Ditran, and ataxia after about 280 min.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

Gershon and Angrist (247) summarized the research that had been done with Ditran and other anticholinergic psychotomimetic compounds and with possible antagonists of their psychotomimetic activities. Nothing particularly new was reported in this paper. Tacrine is regarded as the best overall antagonist.

Sayers and Burki (71) compared the effectivenesses of 13 psychoactive compounds, including Ditran, in four assays of anticholinergic potency: displacement of 3-quinuclidinyl benzilate from the particles in a homogenate of rat brain, antagonism of stimulation of isolated guinea pig ileum by acetylcholine, antagonism of induction of tremor by oxytremorine in mice, and production of pupil dilatation in mice. Ditran was graded first in the first two tests, third in the oxytremorine test, and second in the mydriasis test. For comparison, atropine was as good as Ditran in the second test, first in the last two tests, and second in the first test. Overall, therefore, Ditran was judged to be slightly less anticholinergic than atropine. The general conclusion was that none of these tests is satisfactory for predicting in vivo effects on brain functions.

Kligman and Copelan (156) reported that 16 volunteers had been used for studies with Ditran, but gave no information about the studies.

Ketchum et al. (24,165,166) gave 22 male volunteers intramuscular Ditran at 50-170 µg/kg and concluded that 150 ug/kg produced a syndrome of peripheral parasympatholytic effects, disturbances of basic neuroregulatory functions, and disruption of higher integrative functions that culminated in delirium characterized by restlessness, confused speech, hallucinations, and moment-to-moment variability. Twelve of these volunteers were treated with physostigmine and three with Tacrine after development of toxic psychosis. Both inhibitors of cholinesterases were found to be effective in overcoming changes in the Number Facility Test induced by Ditran.

3-QUINUCLIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (EA 3167)

The phenylcyclopentylglycolic acid ester of 3-quinuclidinol was included in the group of glycolic esters studied by Larsson et al. (192). They recorded its threshold dose for producing a psychotomimetic effect in the dog as 50 ug/kg. Five substances with threshold doses of 10 ug/kg had hydrogen bond strengths (expressed as log K, where K is the constant for the equilibrium between free and bonded CO groups in infrared spectra of the glycolates) of 0.8-1.2 (mean, 1.01). Two substances with threshold doses of 50 µg/kg, one being Ditran, had log K values of 0.80 and 0.93; two other substances with threshold doses of 0.5 mg/kg, one being tropinyl benzilate, had log K values of 0.92 and 1.2. It is evident that there is no correlation between the strength of the intramolecular hydrogen bond and the threshold dose for psychotomimetic effect.

Ketchum et al. (248) gave six volunteers intramuscular 3-quinuclidinyl-(phenylcyclopentyl)-glycolate by intramuscular injection at 2.4 µg/kg, seven at 2.9 µg/kg, and six at 3.4

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

µg/kg (the hydrochloride salt was used). Measurements and observations of the subjects were made hourly for 6 h, bihourly for another 6 h, and every 4 h thereafter until recovery was judged to be virtually complete. The MED_{50} was found to be 2.5 µg/kg, and the ID_{50} was estimated to be 4.1 µg/kg. With the latter dose, mild effects were to be expected after 2 h and severe ones after 5 h. Severe effects would be expected to last for about 96 h, and mild ones for about 240 h. Eighteen of the subjects were examined for evidence of organic changes, and 13 were given psychologic evaluations about 6 mo after they had received the compound.

Most subjects displayed some change from baseline performance and behavior when examined 2 wk after injection of the glycolate; these changes usually disappeared by the fourth week after the injection. The effects detected in these early followup examinations included increased irritability; mild impairment of memory, judgment, or abstraction; mental sluggishness with occasional confusion; nervousness; and tenseness. No positive evidence of organic change was found at 6 mo after the doses; there may have been a small increase in IQ at that time. The profiles in the MMPI of seven subjects who were tested 6 mo after exposure, as well as before exposure and a month after exposure, were close to the baseline profiles, although a month after exposure they had contained significant increases in the scores on the hypochondriasis, depression, hysteria, psychasthenia, schizophrenia, and mania scales. Intoxication with the glycolate was found to be treatable with physostigmine or Tacrine; because of the long duration of the effects in some subjects, doses of antagonists may have to be repeated for a rather long time.

L-2-ALPHA-TROPINYL-L-(PHENYLCYCLOPENTYL)-GLYCOLATE (226,086)

The actions of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate on experimental animals have been described (188). This was found to be the most active and the safest (largest ratio of LD_{50} to MED_{50}) of the six possible isomers of tropinyl-(phenylcyclopentyl)glycolate by intravenous injection into mice. LD_{50}s by intravenous injections into male mice and rats were 61 $\pm$ 3 and 65 $\pm$ 3 mg/kg, respectively; the LD_{50}s by gavage in the same two species were 98 $\pm$ 21 and 170 $\pm$ 53 mg/kg. Depression of general motor activity and depression of respiratory activity were the principal effects observed in the two test species during the estimations of LD_{50}s. A range-finding test with intravenous injection of the glycolate into monkeys yielded an LD_{50} probably between 10 and 20 mg/kg. In the cat, the LD_{50} probably is greater than 20 mg/kg.

L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate elicited mydriasis in the mouse after intravenous doses of 10 µg/kg or more. One estimate of the MED_{50} was 5.6 $\pm$ 1.5 mg/kg. Mydriasis became maximal after intravenous doses of about 0.3 mg/kg. Other effects that were evident after doses of 10-30 ug/kg were hyperactivity, hyperreactivity to touch, and motor deficits. Respiratory depression became evident

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

after intravenous doses of 0.3-3.0 mg/kg. General activity became lessened and ptosis of eyelids appeared after doses of about 30-50 mg/kg. Mydriasis was the most persistent effect, which might last for 3-4 d after the highest doses mentioned above. With mydriasis as the indicator, this glycolate was about one-third as active when applied to the skin of the mouse as when injected subcutaneously (benzyl alcohol was a suitable solvent for application of the glycolate to the skin). Intravenous doses of about 50-100 µg/kg depressed a polysynaptic reflex (ipsilateral flexor reflex) in the anesthetized cat, but did not affect the patellar reflex. This glycolate was effective in antagonizing tremor in mice (induced by subcutaneous injection of tremorine at 10 mg/kg), in subcutaneous doses of 10-30 µg/kg whereas, by the same route of administration, doses of 12-16 µg/kg were effective in inducing mydriasis. The glycolate had a dose-related negative inotropic effect on the isolated, perfused rabbit heart, with only a slight negative chronotropic effect. In the intact anesthetized dog, it had dose-related negative effects on both heart and mean blood pressure. L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate was active prophylactically against 2 LD_{50}s of TEPP (2.3 mg/kg intraperitoneally) in mice pretreated by intraperitoneal injection of benzoquinonium at 0.3 mg/kg (to prevent neuromuscular paralysis by TEPP), its intraperitoneal ED_{50} being 40 ug/kg.

Rats given five daily intravenous injections of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate at 50 mg/kg during 8 d and rats given intravenous injections 5 d/wk for 1 mo at 10 mg/kg had no changes observable at necropsy, except decreases in the sizes of the spleen and the thymus in the animals given 50 mg/kg. Histologically, both groups of rats had increased numbers of granules in the Paneth cells in the crypts of Lieberkühn in the small intestine. Two of five rats given the largest dose were thought to have slight depletion of thymocytes.

Monkeys given daily intravenous injections of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate at 0.4, 2.0, or 10 mg/kg 5 d/wk for a month had mydriasis with all doses. Tremors occurred in the animals given the two larger doses, and convulsions in those given the largest. Various extents of involution of the thymus and of increased granule formation in the Paneth cells were seen in animals given each of the doses. Fatty changes were seen in the livers of some of the monkeys given the two larger doses. One of five monkeys given 0.4 mg/kg died on the twelfth day, and one of five given 10 mg/kg died on the twentieth day. No specific cause of death was identified in either instance, but both animals had lost more weight than the means of the living members of their groups.

The actions of this glycolate in unanesthetized beagles were studied (249). Groups of four dogs were given daily intravenous injections at 0, 0.8, 4.0, and 20.0 mg/kg 5 d/wk for a month, receiving 21 doses during 29 d. Each group contained two males and two females. Three of the dogs given the highest dose were found dead on the fourteenth,

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

seventeenth, and twenty-sixth days; the fourth dog in that group was moribund and was killed on the twentieth day. No animals from the other groups died during this study.

All dogs that received the glycolate had mydriasis and reduced activity, the mydriasis persisting even over weekends. In the high-dose group, panting, persistent anorexia, tremor, ataxia, prostration, fits of running movements of the legs (possibly accompanied by barking) in prostrate animals, and convulsions were seen commonly. These dogs lost an average of at least 23.5% of their body weight before death. The dogs of the groups given the low and medium doses of the glycolate lost 4.4% and 10.8%, respectively, of their original body weight during the administration of the glycolate. The heart rates of the dogs given the glycolate all increased during the first 15 min after administration of the first doses, whereas those of the control animals, given intravenous injections of saline, decreased slightly on the average. After the first weekend, after three injections during the previous week and before injection of another dose of the benzilate, the heart rates of the animals given the two higher doses were still above their baseline values. Baseline heart rates were not available for the dogs given the lowest dose, but the heart rates of two of the four dogs in this group on day 6 were above the highest baseline rate recorded for the other 12 dogs in the experiment. On the twenty-ninth day of the experiment, at 2 h after the last doses of saline or glycolate, the mean heart rates, in comparison with those measured at the same time after the first doses, for the three groups of dogs then alive were as follows: control, 9 beats/min below that on the first day; low dose, 29 beats/min below that on the first day; and medium dose, 43 beats/min below that on the first day.

The composition of the blood (blood chemistry and hematology) changed significantly only in the group of dogs given the largest dose of the glycolate. Final samples of blood were obtained on only two of the four dogs in this group; in both, the prothrombin time was prolonged, the total serum cholesterol concentration was very low, and the alkaline phosphatase activity in the serum was very high (70 and 198 K-A units/100 ml vs. 4-12 in baseline measurements). Both dogs had other evidence of interference with liver function: increases in the activity of glutamic-oxaloacetic transaminase and in the concentration of serum bilirubin in one, and decreases in the concentrations of albumin and total protein and in the ratio of albumin to globulin in the serum of the other. The first of these two animals had a concentration of serum urea nitrogen that was above the normal range, so there seemed to be some interference with renal function also. The most striking change in the hematologic picture in these two dogs was the complete eradication of eosinophilic polymorphonuclear cells from the blood. The total leukocyte counts and hematocrits were decreased in both dogs; one also had a moderate decrease in erythrocyte count.

Terminal samples of urine were obtained from the bladders of all four of the dogs in the group given the highest dose of the glycolate. Two were bloody, and three contained bilirubin,

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

bile salts, and urobilin. All four samples contained albumin. The urine of the dog mentioned above as having increased serum urea nitrogen contained hyaline casts.

Electrocardiographic recordings from the dogs given the medium dose of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate contained suggestions of deepening of the S wave and of elevation of the T wave. Because baseline recordings of the ECG had not been made for these animals, the significance of the apparent changes cannot be assessed.

The only significant pathemas found in the dogs at necropsy at the end of the experiment were in those given the highest doses of the glycolate. The livers of these dogs were pale yellow to orange and were coarsely lobular or swollen. Three of these dogs had bile staining of the sclerae and aortas. Two of the dogs had tarry material in their gastrointestinal tracts, reddish urine in their urinary bladders, and fatty bone marrow. Hemorrhages into the renal cortices were found in these two dogs. Microscopic examination of organs from these dogs revealed decreases in the size and number of the islets of Langerhans in one dog from each of the low-dose and high-dose groups and in two dogs from the medium-dose group. In the high-dose group, irritative and degenerative or necrotic changes were also found in the livers, kidneys, bone marrow, adrenals, testes, epididymides, and gastrointestinal tracts. The livers of all the dogs in this group were markedly fatty, the hepatocytes containing numerous small cytoplasmic vacuoles. Widespread vacuolation of the tubular epithelium in the cortical region of the kidney was seen in three dogs; epithelial crescents were seen in Bowman's spaces in the kidneys of two of these dogs. The bone marrow of all four dogs had shifted to an immature type, with increased numbers of blast forms of the granulocytic series.

2-METHYL-3-QUINUCLIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (301,060)

Hayes (250) reported the results of a study in which 29 men were given single intravenous doses of CAR 301,060, also called cis-2-methyl-3-quinuclidinyl-(phenylcyclopentyl)-glycolate, at 0.5-5.3 ug/kg . They were graded in performance in the Number Facility Test. Two subjects given 5.3 ug/kg became incapacitated (two consecutive scores in the Number Facility Test below 10% of baseline); the mean duration of significant effect was 73 h. These two subjects were disoriented, confused, restless, and less coordinated than normal, spoke disjointedly and in a slurred manner, had lapses of memory, and experienced visual hallucinations. One was episodically paranoid and mildly hostile to the medical personnel in attendance. Both were drowsy and lethargic. Their heart rates were increased markedly for more than 24 h after injection, but the peak of the tachycardia (+85%) occurred about 12 h after injection. This dose was the only one that resulted in pupil dilatation, by about 2 mm.

Doses of 2.0 and 2.7 ug/kg produced only slight and irregular increases in heart rate. The heart rate was increased in a dose-related manner when doses of 3.2 ug/kg and

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

more were administered. The lowest dose that produced a significant decrease in the performance of one of four subjects in the Number Facility Test was 2.7 µg/kg; 3.2 µg/kg produced significant decrements in the performances of five of seven men, and 3.8 µg/kg caused significant decrements in the performances of six of eight subjects. Hayes concluded that the intravenous MED_{50} of this glycolate for man is about 3.0 µg/kg and that, after a dose of 3.2 µg/kg, a significant decrease in cognitive ability occurs about 5 h after injection and lasts for about 7 h.

ESTERS OF PHENYLISOPROPYLGLYCOLIC ACID

Two esters of phenylisopropylglycolic acid are among 26 anticholinergic substances studied by Edgewood Arsenal: N-methyl-4-piperidinyl-(phenylisopropyl)-glycolate (EA 3834) and 4-(1-methyl-1,2,3,6-tetrahydropyridinyl)-phenyl-isopropylglycolate (302,668).

N-METHYL-4-PIPERIDINYL-(PHENYLISOPROPYL)-GLYCOLATE (EA 3834)

Copelan (251) gave 16 volunteers EA 3834 at 1.0-2.7 µg/kg intravenously and administered the Number Facility Test to them at intervals thereafter. Two groups of two men given 1.0 or 1.4 µg/kg had their performances in the Number Facility Test unchanged. Some members of each of two groups of four men given 1.6 or 2.0 µg/kg had their performances reduced to below 75% of their baseline performances. Three of four men given 2.7 µg/kg had their performances reduced to below 75% of the baseline. The estimate of the MED_{50} was 1.96 µg/kg, with 95% confidence limits of 1.45 and 2.66 µg/kg. Significant change in performance in the Number Facility Test usually began at about 1.5 h at the highest dose administered (2.7 µg/kg), the mean time of onset of significant effect on performance was 1.9 h, and the duration of significant effect was 2.8 h.

Copelan described the effects of this agent as follows. At 3-15 min after injection, the subjects reported that they felt "high," "light-headed," or "dazed." At about this time, they were unsteady in walking and particularly in turning about. Talking among the subjects became quieter, and their speech was slightly muffled, but without significant slurring. A feeling of heaviness of the eyelids and a tendency to diplopia became evident at 10-15 min after injection. Sedation, with drowsiness and dozing, were noticeable at 15-30 min. One subject (dose group not stated) reported an illusion of undulating movement in the glass of a window. Two subjects (dose groups not identified) reported hallucinations of crawling ants and of moving strings or hairs on the floor, respectively. The same two subjects recognized that they were hallucinating. There was no confusion, significant disorientation, or delirium among the subjects in this study. dryness of the mouth, blurred vision, tachycardia, and mild reddening of the conjunctivae were noted occasionally and might appear within 15-30 min after injection. No abnormal changes in blood and urine were detected 24 and 48 h and 8 and 28 d after injection.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

Averill et al. (252) summarized studies of the toxicity of EA 3834 in mice, rats, rabbits, cats, dogs, and monkeys. Table 3 gives the available single-dose LD_{50}s for both EA 3834 and 302,668. It is apparent that the latter is less lethal than the former in the species for which values on both compounds are available. The values for 302,668 come from Sim (253).

In addition to the LD_{50} values stated above, Averill et al. summarized the results of studies in which graded doses of EA 3834 had been administered to various animals by a variety of routes. In mice, intravenous injections at less than 10 ug/kg induced mydriasis and those of 32-100 µg/kg induced unusual sensitivity to being touched, restlessness, and ataxia. Intramuscular doses of 100 mg/kg in the rat induced paralysis of the leg into which the compound was injected within 5 min after injection and jerking movements of the head, hyperpnea, and exophthalmos within about 15 min after injection. Intravenous doses of 0.7 µg/kg induced mydriasis in rabbits, and tachycardia followed doses of 10-100 µg/kg. Ataxia in this species appeared after intravenous doses of 3 mg/kg, and prostration and convulsion occurred after doses of 11-14 mg/kg. In the cat, mydriasis, decreased activity, increased heart rate, and ataxia all occurred after intravenous doses of 10-100 µg/kg.

The heart rate of the dog was increased and the performance of a conditioned-avoidance task was degraded by intravenous doses of 10-28 µg/kg. Doses of 12-37 µg/kg reduced physical activity, and those of 25-30 µg/kg induced ataxia. Prostration and convulsions occurred after doses of 10-20 mg/kg. Inhalation exposure of dogs to a Ct product of EA 3834 of 36 $mg.min/m^3$ resulted in increases in heart rate. Physical activity was reduced by exposure to Ct products of 40-50 $mg.min/m^3$. Mydriasis and ataxia followed exposure to a Ct product of 90 $mg.min/m^3$. A Ct product of 102 $mg.min/m^3$, with 95% confidence limits of 82 and 126 $mg.min/m^3$, caused 50% of the exposed dogs to fail in performance of the conditioned-avoidance task. In the monkey (species not stated), intravenous doses of 6 µg/kg induced mydriasis. Visual discrimination was reduced by doses of 7-31 µg/kg. Tachycardia and decreased activity followed doses of about 130 µg/kg. Prostration and convulsions were produced by doses of 14-16 mg/kg.

Sidell and Braun (254) discussed the results of an experiment in which two men were given EA 3834 by mouth at 7 µg/kg, four were given 10 µg/kg, three were given 14 µg/kg, and two were given 20 µg/kg. One man given 7 µg/kg and two given 10 µg/kg suffered severe effects (reduction of score in the Number Facility Test to below 25% of the baseline score). All the men given 14 and 20 ug/kg experienced severe effects.

Of those who experienced severe effects, the three with the lower range of doses had a mean dose of 9 µg/kg and experienced severe effects at about 2.3 h after drinking a solution of the agent in water. The five men with the higher range of doses had a mean dose of 16.4 µg/kg and experienced severe effects at about 1.4 h after ingestion. The two men given 7 µg/kg had a mean score of 47% of their baseline

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

scores. The four men given 10 µg/kg had a mean score of 40.4%. The five men given a mean dose of 16.4 µg/kg had a mean score of 2.6%. The eight severely affected men had recovery times of 12-16 h (mean, 12.5 h). The two men given 10 µg/kg who did not suffer severe effects had recovery times of 6 and 10 h. The severe effects lasted 0.5-9.0 h; the mean duration of severe effects for the three men who took a mean dose of 9 µg/kg was 3.3 h, whereas that of the five men who took a mean dose of 16.4 µg/kg was 6.4 h.

Mild effects (reduction of score in the Number Facility Test by at least 25% of the baseline score) for all the severely affected subjects lasted 10-14.5 h (mean, 11.2 h), whereas those in the two men who took 10 µg/kg without developing severe effects lasted for 3 and 7.5 h. Mild effects in the two men given 10 µg/kg who never developed severe effects began 2.5 and 3 h after ingestion. In the eight men who developed severe effects, the mean onset time for mild effects was 1.3 h (95% confidence limits, 1.0 and 2.0 h).

McCarroll et al. (255) described a study with EA 3834 in which eight enlisted volunteers from the U.S. Army were divided into two four-man teams, with a ninth serving as the leader of both groups. The ninth man had been given intramuscular scopolamine at 23.5 µg/kg several weeks earlier to familiarize him with the effects that the others would experience later, but he was not given an anticholinergic substance during the actual study. The members of the two teams were given directions for performing a simulated military mission in the field and then practiced the mission for 3 d. On the fourth day, the teams went through the entire mission under the observation of two judges, who graded their performances on a number of items incorporated into the exercise. The exercise included capture and interrogation by a simulated aggressor. On the day of the test, intramuscular injections were given at 9 a.m., and the exercise was started immediately. It ended at 4 p.m. A battery of tests (heart rate, blood pressure, rectal temperature, Number Facility Test, and behavioral checklist) was administered to each volunteer at 9:30, 10, and 11 a.m., noon, and 1 and 4 p.m. The performance of volunteers on this day was graded by the same judges who had been present on the control day.

One four-man team received EA 3834 at 2 µg/kg (one man), 4 µg/kg (two men), and 8 µg/kg (one man), for a mean dose of 4.5 µg/kg. The second four-man team received 4 µg/kg (one man), 8 µg/kg (two men), and 12 µg/kg (one man), for a mean dose of 8 µg/kg. Both teams were considered to be composed of capable, effective soldiers on the control day.

On the test day, the team with the lower mean dose was judged to be marginally effective. The man given 8 µg/kg had to be removed from the exercise about 2 h after injection. The other members of that team stumbled and fell over minor impediments in the terrain, lost some of their gear, made serious errors in calculating an azimuth with compasses, were unable to remember simple directions or to repeat instructions given just before questioning, and were unable to operate a field telephone. The man given 8 µg/kg cooperated with the

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

"enemy" interrogator and tried to follow his instructions without intellectual or physical resistance. The team that received the larger mean dose became totally ineffective within about an hour. The man given 12 µg/kg became incapacitated within about 30 min and was removed from the exercise. One of the men given 8 µg/kg became incapacitated about 75 min after injection, and the other became incapacitated about 141 min after injection. The man in this group given 4 µg/kg was not incapacitated, but the team leader had to prod him constantly to get him to accomplish the simplest tasks. For both groups, the correlation between performance in the Number Facility Test and in the various military tasks included in the scenario for the field exercise was good.

The volunteers evacuated from the field exercise were flushed and had hot, dry skins, parched lips and tongues, dilated pupils, tachycardia, and exaggerated deep tendon reflexes. After treatment with physostigmine salicylate (55 µg/kg intramuscularly), these volunteers were restored to lucidity within 20 min. After institution of maintenance doses of physostigmine salicylate by mouth, the subjects were able to return to the field exercise and to perform their duties capably. When the maintenance doses of physostigmine salicylate to the volunteer who had been given EA 3834 at 12 µg/kg were discontinued after four such doses, he became delirious again within about 3 h and remained in that state for 5 or 6 h. No abnormalities of renal and hepatic functions were detectable in any subject 24 h and 7 d after the injections of EA 3834.

Cucinell et al. (256) applied EA 3834 in a solution in ethanol to the skins of 11 volunteers on single occasions, of three volunteers on two occasions, and of two volunteers on three occasions. In all instances, the area of skin to which the solution was applied was about 9 cm^2 of the neck at the angle of the jaw. When the solvent had evaporated, the area of application was covered with a plastic cup or with Teflon taped to the skin. The contaminated skin was washed with alcohol 4 h after the application. Some of the volunteers were placed in a hot room at 40.5°C and 24% relative humidity with a wind speed of 0.5 m/s (slightly more than 1 mph); others remained under unspecified room conditions. Three of the volunteers who received EA 3834 more than once were exposed to it both in the hot room and in the regular laboratory rooms; the other two in this category were exposed only in the hot room. The amounts of the agent applied to the skin ranged from 0.1 to 5.0 mg in the hot room and from 2.0 to 4.0 mg in the regular laboratory rooms.

The hydrochloride of EA 3834 had no measurable effect on rate of sweating or performance in the Number Facility Test. When EA 3834 base was applied to the skin, the time to onset of inhibition of sweating (38-170 min) in the hot room was related inversely to the amount of the agent placed on the skin within the range 0.1-3.0 mg. The rate of change of the rate of sweat secretion once inhibition of that rate had begun was somewhat less variable (13-42 $mg/m^2.min^2$) within the same range of amounts of the agent, but was randomly distributed throughout

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

the range. The two extreme values of the rate of change of the rate of sweating came from experiments in which sequential amounts of the agent near the middle of the range were applied to skin, the larger of the two amounts producing the smaller rate of change. Because the earliest time after application of EA 3834 to the skin at which the Number Facility Test was administered to the volunteers was 4 h, there is no factual basis for the assumption by Cucinell et al. that there is a constant relationship between the time of onset of inhibition of sweating and the extent of decrease in performance in the Number Facility Test. Their proposed expression for estimation of the systemic effect of contamination of skin with this agent depends on this spurious relationship and cannot be considered reliable.

4-(1-METHYL-1,2,3,6-TETRAHYDROPYRIDINYL)-PHENYLISOPROPYLGLYCOLATE (302,668)

Copelan (257) summarized experiments in which 20 volunteers were given 302,668 intravenously at 1.0-4.6 µg/kg and were tested at intervals for ability to perform in the Number Facility Test. Men given doses of 1.0, 1.4, and 2.0 µg/kg lost less than 25% of their baseline abilities to perform the additions. Of four men given 2.7 µg/kg, two lost at least 25% of their abilities. Of four men given 3.2 µg/kg, one lost 29% and another lost 24%. Four men given 3.8 µg/kg and two given 4.6 µg/kg had mean decrements of 41% and 50%, respectively, and all lost at least 25% of their abilities.

Karger (258) estimated the ID_{50} of 302,668 by giving 14 volunteers intravenous doses of 5.0-11.0 µg/kg and administering the Number Facility Test at intervals thereafter. Incapacitation was defined as the making of two consecutive scores below 10% of the baseline score. A clinical evaluation of incapacitation based on observation of the subjects was also made. The scores on the Number Facility Test yielded an ID_{50} of 10.1 µg/kg, with 95% confidence limits of 9.2 and 11.1 µg/kg. The clinical evaluations yielded an estimated ID_{50} of 9.5 µg/kg, with 95% confidence limits of 8.5 and 10.6 µg/kg.

As a second part of the same study, Karger gave six volunteers 302,668 intravenously at 10 µg/kg and administered physostigmine at various times. An intramuscular dose of 3 mg of physostigmine injected 10 min before administration of 302,668 attenuated the decrement in performance in the Number Facility Test for about 4.5 h after injection. At that time the performance of two unprotected subjects and of the two prophylactically protected subjects became essentially identical. When a dose of 3 mg of physostigmine was injected intramuscularly 10 min after intravenous injection of 302,668, there was rapid recovery from about 35% of baseline performance to about 92%, but performance in the Number Facility Test was down to about 20% of baseline by 2 h after the injection of 302,668. When the physostigmine was not injected until 45 min after the agent, performance in the Number Facility Test rose from zero to about 85% within about 45 min and to a peak of

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

about 93% within 75 min. Performance in the test oscillated between 93% and 70% of baseline until about 3.75 h after injection of the agent and then drifted down to about 40% at 8 h. Thereafter, it improved gradually without further administration of physostigmine; thus, performance in the test had improved to about 60% of the baseline value by 10 h after initiation of intoxication. Karger concluded that the best results in maximally incapacitated persons would be obtained by giving repeated intramuscular doses of 2 or 3 mg of physostigmine at intervals of about 2 h.

Sidell et al. (259) estimated the incapacitating dose of 302,668 inhaled as an aerosol. Performance in the Number Facility Test was the criterion of incapacitation. Nineteen volunteers inhaled aerosols of the agent while seated and wearing an oronasal mask, so that the expired air could be collected and analyzed for unretained agent. Four ranges of retained dose of the agent were recognized: 1.99-7.60 µg/kg, five volunteers; 8.3-11.54 µg/kg, four volunteers; 12.36-12.95 µg/kg, six volunteers; and 13.05-18.77 µg/kg, four volunteers. The mean performances in the Number Facility Test of all groups fell below the level for minimal effect (score less than 75% of baseline score), but that for the last group was the only one that fell below the level for incapacitation (score less than 10% of baseline score). The mean performance of the last group was below the criterion for incapacitation from about 0.25 h to about 5.5 h after exposure. That group also underwent marked decreases in its scores on an Orthorator for near and far vision, in its ability to move pegs in a pegboard, and in its performance of a bend-twist-touch task, whereas the other three groups had comparatively minor changes in these four measurements.

The initial signs and symptoms induced in these volunteers consisted of dryness of the mouth, drowsiness, and ataxia; these were followed by hallucinations, marked confusion, poor coordination, restlessness, and prolonged impairment of accommodation for both near and far vision with the larger retained amounts. Retention of large amounts (15 µg/kg) sometimes resulted in fine fasciculations of muscles. One subject in each of the two highest ranges of retained agent vomited, and eight others, including subjects from all ranges of retained agent, complained of nausea. The heart rate was not altered significantly among the volunteers in the three groups with the lowest ranges of retention, but three of four subjects in the other group had heart rates above 100 beats/min. No consistent effects on blood pressure were observed. Pupil dilatation occurred in all groups; in some subjects among those with the higher retentions, mydriasis persisted for 72 h.

From the results of these exposures, Sidell et al. (259) calculated that the ICt_{50} for a man breathing 10 L/min would be 181 $mg.min/m^3$, with 95% confidence limits of 159 and 206 $mg.min/m^3$. For a man exercising moderately and breathing 15 L/min, the ICt_{50} would be 121 $mg.min/m^3$, with 95% confidence limits of 106 and 137 $mg.min/m^3$. The incapacitating dose of retained agent for 50% of exposed men,

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

the IRD_{50}, was calculated to be 13.1 µg/kg, with 95% confidence limits of 11.1 and 15.3 µg/kg. With this retained dose, the time to onset and the duration of mild effects were expected to be 25 min and 12 h, respectively; the times for severe effects were predicted to be 30 min and 5 h, respectively.

Lavallee (50) collected and graphed data from human experiments that indicated that 302,668 at comparatively low doses (below 4 µg/kg) approached 3-quinuclidinyl benzilate in activity in decreasing performance in the Number Facility Test, but at higher doses (above 9 µg/kg) was only slightly more active than scopolamine. Extrapolation of Lavallee's graphs suggests that the doses of 302,668 and of scopolamine that would be able to lower performance in the Number Facility Test to zero would be identical.

Sim (253) summarized the body of research with 302,668, printed or unpublished, performed in 1964-1971. The first one-third of the report discussed the chemical and physical properties of 302,668. The last two-thirds summarized the studies performed to determine the actions of 302,668 and several other anticholinergic substances in animal and human subjects. As shown in Table I-3, 302,668 was most lethal to the rabbit and least to the monkey after intravenous injection. It was most mydriatic in the rat and least in the monkey. The species with the safest relation between the lethal dose and the mydriatic dose was the rat, followed by the dog. Species with much smaller values of the ratio of LD_{50} to ED_{50} were the mouse, the rabbit, the cat, and the monkey. Although 302,668 had approximately the same actions as other anticholinergic compounds, the only sort of activity at which it excelled was the inception of increased motor activity in the mouse. The ICt_{50} in humans had been found to be 121 $mg.min/m^3$, which was larger than those for 3-quinuclidinyl benzilate, EA 3580, and EA 3834. Amiton, diethyl-S-(diethylamino)-ethylphosphorothiolate, was mentioned as having been found to be a more potent and longer-lasting antagonist of the autonomic effects of 302,668 in cats than physostigmine.

ESTERS OF 1-PROPYNYLCYCLOPENTYLGLYCOLIC ACID

This group of glycolates includes two compounds: the esters of the subject acid with 3-quinuclidinol (302,212) and with N-methyl-4-piperidinol (302,196).

Copelan (260) gave 25 volunteers 302,212 intravenously at 1.0-4.6 ug/kg. The initial effect experienced by the subjects was a feeling of "light-headedness" or of mild "dizziness" that started 15-30 min after injection. Although this symptom was reported by the subjects to be especially noticeable when they were standing, it was not associated with orthostatic hypotension. Between 30 and 60 min after injection, the subjects became drowsy. They often fell asleep; when walking, they staggered mildly and often brushed against corridor walls. Speech became muffled and slow, but was not slurred. Slight impairment of logical thought and of performance in the Number Facility Test became evident about an hour after

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

injection. The doses used in this study did not produce incapacitation. Peripheral effects were limited usually to dryness of the mouth and, after the higher doses, mild blurring of near vision. The compositions of blood and urine and the removal of Bromsulphalein from the blood were within normal limits at 24 and 48 h, 8 d, and 4 wk. The MED_{50} for inducing at least a 25% reduction in the score in the Number Facility Test was estimated to be 3.3 ug/kg. The latency of the effect with this dose was expected to be about 4 h, and the duration of the effect was estimated to be about 4 h. The latency of the effect changed little, if at all, as the dose was increased, but the duration of the effect increased irregularly, but rather definitely, after the larger doses.

Kligman and Copelan (156) stated that 25 subjects had been exposed to 302,212, but gave no information about the experiments. The 25 subjects probably were those used by Copelan (260).

Lavallee (50) collected data from studies of the effects of 302,196 on human subjects that enabled him to draw a line relating the effects on the score in the Number Facility Test to the logarithm of the dose. This ester was less effective than scopolamine in decreasing the ability to perform this test.

Sidell et al. (261) injected 302,196 into 10 volunteers intramuscularly at 14-54 µg/kg, bracketing the previously determined intravenous ID_{50} of 22.5 µg/kg. The ID_{50} after intramuscular injection (reduction in score in the Number Facility Test to not more than 10% of the baseline score) was calculated to be 28.9 ug/kg, with 95% confidence limits of 12.0 and 65.5 µg/kg. Therefore, this ester was nearly 80% as active by intramuscular as by intravenous injection. At the ID_{50}, the duration of severe effects was expected to be about 101 min and the total duration of effects to be about 263 min. The latency of onset of severe effects was expected to be only about 12 min after intramuscular injection. The ED_{50} of 302,196 to increase the heart rate by 30 beats/min was calculated to be 23.5 µg/kg. The maximal increase occurred at about 15 min, and the heart rate was close to the baseline value by 4-6 h after intramuscular injection. The mean blood pressure was increased slightly, with a time course similar to that of heart rate. Pupil dilatation was slower both in developing and in disappearing than other changes that followed intramuscular injection. No abnormalities of blood and urine were found at 24 h and 7 d after injection.

N-METHYL-4-PIPERIDYL-(PHENYLCYCLOBUTYL)-GLYCOLATE (EA 3580)

Mongrel dogs unselected for sex were given 20 daily intravenous injections of the hydrochloride of N-methyl-4-piperidyl-(phenylcyclobutyl)-glycolate (EA 3580) at 0.01, 0.1, or 1.0 mg/kg during a 28-d period (262). No detectable effects were observed with the lowest dose. Mydriasis, ptosis, dry mouth, decreased activity, ataxia, and weakness of limbs were observed after the two larger doses. Tolerance to the compound developed during the study.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

A similar experiment was performed (263) with male rats. Mydriasis occurred at the low dose (0.01 mg/kg.d). Tremors occurred at the two higher doses. The rats given the two higher doses had their ratios of adrenal weight to body weight increased significantly. No quantitative information has been made available.

A similar experiment was performed with the free base in place of the hydrochloride (264). The same doses used in the two preceding experiments were administered daily, 5 d/wk, for about a month. All doses induced mydriasis. Fine body tremors also occurred. These two observable effects were the only ones mentioned. Their appearance was rapid after injection, their durations were "relatively short," and they were reproduced each day. The groups given the two higher doses had liver weights and ratios of liver weight to body weight that were significantly larger than those of the control rats.

These three estimations of subacute toxicity indicated that a modest number of repeated doses of EA 3580 in the form of the free base or the hydrochloride and in amounts of not more than 10 µg/kg should be safe, although possibly disturbing. Typical anticholinergic effects would be expected.

Aghajanian et al. (265) told of giving four volunteers EA 3580 intramuscularly at 1 µg/kg, seven at 1.4 µg/kg, four at 1.7 µg/kg, and five at 2.0 µg/kg. With the production of at least a 25% decrement in performance in the Number Facility Test as the criterion of a significant effect, the MED_{50} was calculated to be 1.47 µg/kg. After a dose of 2 ug/kg, significant decrement of performance extended from about 3 to about 12 h after injection; the maximal decrement after this dose (mean of four cases) was to about 60% of baseline performance. All subjects experienced drowsiness beginning about 1-2 h after injection and lasting for at least 6 h. Some of the subjects had mildly impaired intellectual functions, but no delirium or hallucinations. None of the subjects had tachycardia, but two subjects developed distinct bradycardia after unstated doses. There was no complete blockage of sweating, although sweating was reduced. Decreased ability to accommodate for near vision was observed in some subjects, and four subjects (with unstated doses) had spasms of accommodation.

A report (266) that presents the results of administering two unstated doses of EA 3580 by an unstated route to groups of four men has been made available. The subjects were familiarized with the problem-solving task, which involved deciding whether an array of seven letter and number symbols was the same as an array seen previously and then pressing one of two buttons to indicate whether the two arrays were the same or were different. Six days after a baseline session following the familiarization session, the subjects were given EA 3580 and were tested for ability to perform the task with the duration of presentation that had been found to yield a stable accuracy of 65-75% in the familiarization session and that had been used in the baseline session. About all that can be derived from the data presented is that EA 3580 produced a more marked decrease in the rate of responding than in the accuracy

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

of the responses and that the effect was more marked under conditions of extinction (no reward for a correct response) than under those of reinforcement. One group of subjects was affected by EA 3580 more than the other, but the doses are unknown.

Crowell (267) presented an estimate of the incapacitating dose of EA 3580, in the form of the hydrochloride, for man, based on the results of intramuscular injections into 22 volunteers at 2.0–5.3 µg/kg. The criterion of incapacitation was reduction of the score in the Number Facility Test to not more than 10% of the baseline score for three consecutive scores. Doses of 2.0 and 2.7 µg/kg incapacitated none of nine subjects. One of five subjects given 3.8 µg/kg was incapacitated. All eight subjects given 4.5 and 5.3 µg/kg were incapacitated. The ID_{50} was calculated to be 3.9 µg/kg, with 95% confidence limits of 3.6 and 4.3 µg/kg. The duration of incapacitation after the ID_{50} was about 90 min; the duration of severe effects after that dose was estimated to be about 5 h. The time to onset of severe effects was about 2.5 h. No adverse effects of single doses of EA 3580 of the magnitude used in this study on the liver, kidneys, or blood-forming organs were detected. The effects on the volunteers were similar to those of other anticholinergic substances. By 24 h after administration of the largest dose (5.3 µg/kg), the subjects were able to care for themselves. Complete recovery did not occur until 28–52 h after injection, without apparent relation to the dose.

Kitzes et al. (154) found that two groups of men with average body weights of 83.1 and 66.7 kg that had received similar doses of EA 3580 (3.1 and 3.2 µg/kg, respectively) suffered significant decrements in their scores in the Number Facility Test in 45.5% and 53.8% of the cases, respectively. It seems probable that body weight played no important role in conditioning the response. This puts EA 3580 in a class with 3-quinuclidinyl benzilate and distinguishes it from EA 3443 and tropinyl benzilate. EA 3443 seemed to have a positive correlation between body weight and response, whereas tropinyl benzilate seemed to have a negative correlation between body weight and response.

Hart and Balter (238) found that volunteers exposed by the respiratory route to EA 3580 at 4.9–18.6 µg/kg and tested before and at intervals up to 5 wk after the exposure with a modified Army General Classification Test gave no evidence of persistent effects from the exposures.

Baker (268) compared the responses of volunteers to EA 3580 administered as a dose per man or as a dose per unit of body weight. Four indicators of effect were used: accommodation for near vision, arm-hand steadiness, dynamic flexibility, and manual dexterity. The general conclusion was that the use of the dose per unit of body weight may increase variance, rather than control for extraneous sources of variation, when the purpose of a study is to establish the effects of a substance itself. The use of multiple-regression equations was suggested as an approach to the establishment of definitive information on effects of chemicals.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

Kitzes et al. (269) exposed 16 volunteers to an aerosolized 2% solution in acetone of EA 3580 in the form of the free base and subjected them to a battery of tests before and after exposure. The men were exposed to three ranges of concentration of the ester: seven were exposed to concentrations corresponding to doses of 4.9–5.9 µg/kg (mean, 5.4 µg/kg), six to doses of 6.4–13.4 µg/kg (mean, 9.1 µg/kg), and three to doses of 17.0–18.6 µg/kg (mean, 17.9 µg/kg). The lowest range of doses produced no hallucinations or hostile or irritable behavior. The middle range of doses produced hallucinations in four of the six subjects and mild hostile behavior in one. The highest range of doses produced hallucinations and hostile or irritable behavior in all three subjects. In one of these subjects, the degree of hostility induced was considered severe, whereas it was mild in the other two. All the subjects in the last group were incapacitated within 30 min after exposure. All became delirious, and one semicomatose. All the subjects in this group and two of those exposed to the middle range of doses were thought by physicians who were not privy to the details of the experiment to require treatment by injection and oral administration of physostigmine. The toxic delirium disappeared within about 15 min after the beginning of therapy.

The ID_{50} of aerosolized EA 3580 was calculated to be 8.4 µg/kg, with 95% confidence limits of 5.8 and 12.2 µg/kg. The time to onset of incapacitation was about 2 h, and spontaneous recovery from severe effects required about 9 h from their inception. The effects induced by inhalation of aerosolized EA 3580 were the same as those induced by intramuscular injection of this agent. To judge by comparison of the retained ID_{50} for the aerosolized material and the ID_{50} estimated by Crowell (267) for the intramuscularly injected compound, the inhaled compound seems to have only 46.4% of the activity of the injected compound.

Baker (270) added to the previously mentioned volunteers given fixed doses of EA 3580 in the form of its hydrochloride (268) 16 volunteers given intramuscular injections of the estimated ID_{50} for a subject of average weight; 16 other volunteers were control subjects. Nine measures of performance were used. Each volunteer was tested five times during a 25-h period after injection of agent or placebo. The indicators of effect of EA 3580, in order of decreasing sensitivity, were manual dexterity, accommodation for near vision, accommodation for distant vision, arm-hand steadiness, estimation of elapsed time, short-term memory, dynamic flexibility, and static strength.

Baker et al. (158), in a summary report, stated that performance in a series of tests had been shown to be correlated with various personal traits and that changes in these performances caused by EA 3580 were clearly differentially related to the particular subject being tested. If the personal traits of a subject were taken into account, a significant portion of the subject-related variance could be compensated. The opinion was expressed that in this way generalizations about effects of substances that would be

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

independent of the special group of subjects used in the research could be generated.

Allen and Safer (271) compared the temporal characteristics of intoxication induced by intramuscular injections of 0.25 mg of EA 3580 in the hydrochloride form in men resting in the laboratory and in men considerably more active in connection with field testing. The subjects in both situations developed their initial deficits in performance in the Number Facility Test at the same rate, but the subjects in the field test experienced a somewhat smaller deficit and returned toward normal more rapidly. The duration of marked mental incapacitation among the subjects in the field test was about six-tenths that among those in the laboratory.

Lavallee (50) used log dose-effect graphs to demonstrate that EA 3580 was nearly as active as EA 3443 and considerably more active than 3-quinuclidinyl benzilate and four other anticholinergic substances in bringing about decrements in performance in the Number Facility Test. In contrast with the sequence of decreasing potency in human subjects of EA 3443, EA 3580, and 3-quinuclidinyl benzilate, the sequence in dogs performing a multiple-stimulus conditioned-avoidance task was 3-quinuclidinyl benzilate, EA 3443, and EA 3580. In monkeys performing a visual-discrimination and avoidance task, the sequence was EA 3580, EA 3443, and 3-quinuclidinyl benzilate. In a similar task involving visual discrimination and escape by monkeys, the sequence of effectiveness of the two numbered compounds was EA 3580 and EA 3443. It is clear that none of the screening tests with experimental animals had reproduced the sequence of potencies found in the human test. The author of the report suggested that groups of animals larger than the two of each species required for the screening tests might increase the reliability of the results obtained with laboratory animals.

Klapper *et al.* (163) found that, for six men given a mean EA 3580 dose of 1.8 µg/kg by intramuscular injection, the scale of the MMPI yielding the greatest correlation with their performances in the Number Facility Test was that for hysteria. For seven men given a mean dose of 3.5 ug/kg, the scale with the best correlation was that for mania. For the lower dose, the most negative correlation was with the paranoia scale; for the larger dose, the most negative correlation was with the depression scale.

Sidell *et al.* (164) found that intravenous injection of S-(diisopropylaminoethyl)-ethylmethylphosphonothioate into men intoxicated with sufficient EA 3580 to decrease performance in the Number Facility Test to 15% or less of their baseline values removed the decreases promptly and nearly completely. Oral administration of the same phosphonothioate was ineffective. The doses of the theraupetic compound were not stated; oral administration of a different dose of the phosphonothioate might be effective.

Armstrong *et al.* (239) stated that the ED_{50} for EA 3580 in decreasing the number of rewards obtained by rats trained in a sequential-response task was 37.1 µg/kg. The ED_{50} for producing the least detectable mydriasis in rats was 16-30 µg/kg.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

302,282

Kligman and Copelan (156) reported that the 1-methyl-4-piperidyl ester of phenyl-(3-methylbut-1-yn-3-enyl)-glycolic acid (302,282) had been administered to 20 men. The results were reported by Copelan (272), who gave 24 volunteers intravenous doses of 1.0-6.5 µg/kg. Doses of 3.8 µg/kg and less of this agent had only slight effects on performance in the Number Facility Test by the 12 men given these doses. A dose of 4.3 µg/kg decreased the test scores to below 75% of the baseline scores for three of four men. A similar result followed doses of 5.4 µg/kg in four men. Doses of 6.5 µg/kg reduced the scores of all four men given them to below 75% of the baseline scores and reduced the score of one man to 9%. The mean score for this group was 30.5% of the baseline scores. The time to onset of a minimal effect after the MED_{50} of 4.4 µg/kg was estimated to be about 30 min, and the duration of the minimal effect was estimated to be about 30 h. The peak effect usually occurred about 2 h after injection.

The initial symptom was mild "dizziness." Heaviness of the eyelids was a part of the initial experience in many subjects. Mild unsteadiness on standing and walking began between 5 and 15 min after injection. At the same time, speech became muffled and slowed. Sedation, with drowsiness and dozing, started about 15-30 min after injection. About 30 min after injection, degradation of performance in the Number Facility Test began. A few subjects had visual hallucinations, and one had auditory hallucinations also.

Dryness of the mouth or throat was reported by some subjects given the lowest doses and by all subjects given doses of 3.8 µg/kg or more. Mild blurring of near vision and occasional urinary frequency began after doses in the upper part of the MED_{50} range, but never affected all men in a dosage group. Mydriasis was not apparent. Nausea was rare. Conjunctival reddening frequently became evident within the first hour after injection. No significant alterations in blood and urine were found at 24 and 48 h, 8 d, and 4 wk after injection.

Safer (273) gave eight volunteers intravenous doses of 302,282 at 7.5-13.5 µg/kg. Three of the subjects became anxious after injection and two of them became agitated, restless, and passively resistant to experimental procedures. One subject vomited and later developed mild myoclonus, hyperactive reflexes, and indications of effects on the pyramidal tracts (positive Chaddock's reflex and possibly positive Babinski's sign). One subject had a tactually detected arrhythmia, which was not detectable in a later recording of the ECG. All the subjects had increased alkaline phosphatase in their serum 24 h after injection; one subject whose baseline activity of alkaline phosphatase (12 K-A units) was at the upper limit of normal had that activity increase to 18.2 K-A units at 24 h after injection of 13.5 µg/kg. On the seventh day after injection, the activity of alkaline

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

phosphatase in his serum was 11.5 K-A units. Alkaline phosphatase activity in one other subject rose from 7.9 to 12.4 K-A units at 24 h after injection and was 8.4 K-A units on the seventh day after injection. This subject also received a dose of 13.5 µg/kg. Two of the subjects had 5-10 white blood cells per high-power field on the seventh day after injection. No other abnormalities in blood and urine were reported.

Safer (273) concluded that the intravenous ID_{50} of 302,282 is 12.5 µg/kg, but that the compound should not be administered to human subjects at doses greater than 11.5 µg/kg because of the untoward changes mentioned above.

302,537

Brown (274) stated that the ester of 2-propenylcyclopentylglycolic acid with 3-quinuclidinol (302,537) is a potent antimuscarinic compound with profound effects on the central nervous system and with a potency comparable with that of EA 3580 and somewhat greater than that of 3-quinuclidinyl benzilate. The times to onset and extinction of effects were unusually short with this agent. It was judged to have no marked toxic or damage-producing effects in any organism or organ system studied. The doses and organ systems used were not stated. The organisms studied were the rat, the dog, and the monkey. The blood-forming organs, liver, and kidneys were said to have been normal after administration of the substance. Presumably, other organs also were examined for pathemas.

Leib (275) stated that he gave 302,537 intravenously at 1.0-7.5 µg/kg to 18 volunteers. However, Figure 1 of his report contains 21 points at doses of 1.0-5.6 µg/kg; no explanation for this discrepancy was given. Eight men given doses of 2.0 µg/kg or less had scores in the Number Facility Test greater than 75% of their baseline scores. One of three men given 2.8 µg/kg had his score in that test decreased to slightly below 75% of his baseline score. The 10 men given 4.0-5.6 µg/kg had their scores in the Number Facility Test reduced to below 75% of their baseline scores, but the lowest score represented in Figure 1 of the paper was 25%, for a subject given 4.8 µg/kg. The intravenous MED_{50} of 302,537 was estimated to be 3.3 µg/kg, with time to onset of minimal effects of 3 h. After a dose of 4.0 µg/kg, the time to onset of minimal effects was 2.6 h; but after a dose of 7.8 µg/kg, the time to onset of minimal effects was only 12 min. The duration of minimal effects after the largest dose was 43 h. Doses of 5.6 µg/kg and more were reported to cause hallucination and delirium accompanied by restlessness and anxiety. In the one graph for a man given a dose of 7.8 µg/kg, incapacitation, indicated by reduction of score in the Number Facility Test to below 10% of his baseline score, occurred about 90 min after the injection. Intramuscular injection of 3 mg of physostigmine about 7 h after the injection of 302,537 induced a marked, but temporary, improvement in the subject's performance in the Number Facility Test. Improvement lasted for about 2 h. Repetition of this dose hours later, after some

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

spontaneous improvement had occurred, had much the same sort of effect as the first dose, but may have increased the rate of spontaneous recovery.

WIN2299

Fine et al. (276) found that the ester of cyclopentyl-2-thienylglycolic acid and diethylaminoethanol (WIN 2299) antagonized the muscarinic (lowering of blood pressure of the dog by intravenous injection of acetylcholine at 5-10 µg/kg), the nicotinic (increase in blood pressure by intravenous injection of acetylcholine at 300-400 µg/kg into an atropinized dog), and the central nervous system (production of large-voltage cortical discharges in unanesthetized curarized cats by Sarin) effects originated by cholinergic or anticholinesterase compounds. WIN2299 had intravenous and subcutaneous LD_{50}s for the mouse of 80 µg/kg and 460 mg/kg, respectively, according to information supplied by the Sterling-Winthrop Research Institute. Daily intramuscular doses of 29.3 µg/mouse, beginning on the day before irradiation was started and continuing until death, prolonged slightly (and more than atropine) survival of mice subjected to whole-body irradiation with 550 R of xradiation, according to Haley and Rhodes (277). The mortality was still 100%. These authors attributed the prolongation of life to diminution in activity of intestinal smooth muscle by the anticholinergic compound, with consequently slower leakage of infective and toxic entities into the body of the mouse from the intestinal lumen.

Pennes and Hoch (278) administered tablets of WIN 2299 to seven patients. Two patients given doses of 2 mg exhibited principally sedative effects. In addition, one of these people described visual illusions and "hypersensitivity to light and sound." Four patients given 6 mg experienced illusions, delusions, and feelings of unreality. The single patient given 10 mg had full-blown delirium, with complete disorientation and visual and auditory hallucinations. No information was supplied on the time courses of these effects.

Fink (279) supplied some information about the time course of effects of WIN 2299. He gave 2.0-3.2 mg intravenously and found that the subjects began to feel restless and excited by about 10 min after the injection. These initial effects might develop into tense fearfulness with visual illusions and delusions. Complaints of dryness of the mouth were elicited by questioning, but were not made spontaneously. The heart rate did not change unless or until excitement and hyperactivity developed. The effects of these doses of WIN 2299 lasted for 2-3 h.

Grob et al. (147) reported the effects of oral doses of 0.5-2.0 mg of WIN 2299 administered on seven occasions to two volunteers who weighed 46 kg (volunteer 1) and 77 kg (volunteer 2). A dose of 0.5 mg to volunteer 1 produced feelings of fullness and heaviness of the head, of being far away from the immediate environment, and of walking unsteadily with subjective and objective impressions of mental depression, with spells of crying. The same dose given to volunteer 2 produced

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

only a sensation of heaviness of the head that began about 22 min after ingestion of the dose and that lasted for about an hour.

A dose of 1 mg to volunteer 1 produced the same effects as the smaller dose, but to a greater degree. There was no effect on heart rate, pupil diameter, or muscular strength. Effects began about 45 min after ingestion and ended about 5.5 h after ingestion. This dose had marked effects on volunteer 2, beginning 20-40 min after ingestion with feelings of slowed movement of the arms and the legs and of slowed speech and thought. These effects were followed by sensations of swelling of the tongue and face, dryness of the mouth, weakness of the limbs, mild giddiness, and marked mental depression. Objectively, there was obvious mental depression, with listlessness, apathy, asthenia, weeping, poor attention, slowed speech, thought, and response, impaired memory (particularly for events in the immediate past), mental clouding and confusion, inability to perform simple calculations, and drowsiness. The subject's grip strength did not change, but the ability to hold arms or hands raised from a position of rest decreased. There was slight objective unsteadiness in walking and slight asteriotaxic effect. There were visual illusions and a slightly increased activity of tendon reflexes. The heart rate and pupil diameter did not change.

A dose of 1 mg of WIN 2299 to volunteer 1 during a period of taking daily oral doses of 8 mg of TEPP produced the same effects as before, but time to onset was longer (90 min) and the effects were a little less severe than when TEPP was not being taken. On the basis of this experience, two additional volunteers (weights, 66 and 53 kg) who were receiving doses of 13-20 mg of TEPP during 5-10 h were given doses of 1 mg of WIN 2299. Ingestion of WIN 2299 had a slight inhibitory effect on mild symptoms induced by the TEPP, beginning after 20-40 min, but little or no effect on moderately severe symptoms. A second dose of WIN 2299 of the same size as the first taken 45 min after the first dose, or the ingestion of a single dose of 2 mg, had slight to moderate inhibitory activity on moderately severe symptoms induced by TEPP. These changes began about 30 min after ingestion of WIN 2299. The effects of WIN 2299 on the central nervous system were less severe in these subjects than in those who had not received TEPP. Atropine was a better antagonist of the effects induced by TEPP than WIN Z299.

NONESTER ANTICHOLINERGIC COMPOUNDS

Table I-4 contains the information that could be obtained on the single-dose lethal activities of the two substances in this group, benzetimide and mepiperphenidol. Information on the toxicities of these compounds was supplied by Janssen Pharmaceutica and Sharp and Dohme Institute for Medical Research, respectively. Benzetimide is 2-(N-benzyl-4-piperidyl)-2-phenylglutarimide, and mepiperphenidol is 1-(3-hydroxy-4-phenyl-5-methylhexyl)-1-methylpiperidinium bromide.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

BENZETIMIDE

In addition to the information on single-dose LD_{50}s for benzetimide, Janssen Pharmaceutica supplied extensive information on subacute and chronic toxicities of this compound. Rats fed diets containing benzetimide or given daily subcutaneous doses 5 d/wk for 14 wk gained weight at a lower rate than control animals when they were fed a diet containing 10 mg/100 g, but not when they were fed one containing 1 mg/100 g or were given subcutaneous doses of 10 or 80 mg/kg. Daily subcutaneous injections of 1.25 mg/kg did not affect the rate of growth of the rats. In general, organ weights in these animals were below those in control rats. Brain weight was not reduced, however, so its relative weight increased. No pathemas or histopathologic changes were found consistently. Rats fed diets containing benzetimide at up to 10 mg/100 g for 21 mo had no unusual gross mortality due to the compound. Among the rats that died in the control group, males accounted for 37.5% of the deaths; among the animals fed diets containing benzetimide, males accounted for 52.2% of the deaths. Among those fed the diet containing the largest concentration of benzetimide, males accounted for 62.5% of the deaths.

Pregnant rats fed diets containing benzetimide at up to 20 mg/100 g on days 6-15 of pregnancy for one or three generations gave birth to litters of the same size as those borne by control rats and produced no abnormal offspring. Pregnant rats given daily subcutaneous doses of 0.63-160 mg/kg during the first 21 d of pregnancy experienced no striking effects at doses below 10 mg/kg. At 10 mg/kg, but not at 5 mg/kg, there was a decrease in the incidence of implantations to 60% of the females, compared with 90-92% in the controls. With daily doses of 40 mg/kg, one of 20 dams died before parturition, and the incidence of implantations decreased to 10% of the females. At the highest dose (160 mg/kg), three of 20 dams died, and no implantations were found. The weights of the offspring at birth were reduced slightly, but no abnormal offspring were reported.

Pregnant rabbits given benzetimide by gavage at 0.31 or 2.5 mg/kg on days 6-18 of pregnancy had increased numbers of dead and resorbed fetuses (28.5% vs. 7.7% in the control group), and one of 59 offspring born to the rabbits given the largest dose of benzetimide was deformed. These various experiments have demonstrated that benzetimide in the doses used gave no evidence of mutagenic or carcinogenic activity and only questionable evidence of teratogenic activity. High doses of the compound did interfere with reproductive processes.

Janssen and Niemegeers (280), on the basis of tests with prophylactic administration of benzetimide to rats given pilocarpine hydrochloride intravenously at 80 mg/kg, concluded that benzetimide had sufficient action on the central nervous system to justify its consideration as a theraupeutic agent in parkinsonism. They considered also that it was one of only three compounds, in a total of 23 anticholinergic substances examined, that were able at submydriatic doses to prevent lacrimation and salivation induced by pilocarpine.

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

Soudijn et al. (281) examined the binding of [^{3}H]benzetimide to subcellular fractions of the rat caudate nucleus and found both in vitro and in vivo that the (+) isomer was bound to the particles in a fraction composed of membranes and nerve endings to the extent of 4-7 times the (-) isomer. Fractions composed more nearly exclusively of membranes or of nerve endings bound the labeled material less extensively than the fraction that contained both structures. Binding of benzetimide in vitro was reduced markedly by atropine and by carbachol, but only slightly, if at all, by d-tubocurarine.

Beld and Ariens (282) measured the binding of benzetimide to microsomes from smooth muscle isolated from bovine tracheae and to particles in homogenates of bovine caudate nuclei. Plotting the amount of [^{3}H]benzetimide bound to the microsomes as a function of the concentration of [^{3}H]benzetimide in contact with them yielded a biphasic curve for (+)benzetimide, whereas that for (-)benzetimide was rectilinear. At a benzetimide concentration of about 6.3 nM, 9 times as much of the (+) isomer was bound to microsomes from vascular smooth muscle as of the (-) isomer. The particles in a homogenate of caudate nucleus bound about 5 times as much of the (+) isomer as of the (-) isomer. The saturable component of the material that bound (+)benzetimide had a higher affinity for the isomer than the nonsaturable component, which bound (-)benzetimide about as well as it bound the (+) isomer. When the microsomes were exposed to a large concentration of unlabeled (+)benzetimide before addition of [^{3}H]-labeled (+)benzetimide, binding of the labeled isomer seemed to be only to the unsaturable component. Atropine was found to bind to the same entities as benzetimide and to block binding of [^{3}H](+)benzetimide by the saturable component of the microsomal preparation.

Karger (283) gave 17 volunteers benzetimide intravenously at 1.0-10.0 µg/kg. He estimated that the MED_{50} for lowering the score in the Number Facility Test to 75% of the baseline value for 50% of a group of subjects was 8.9 µg/kg. All men who met this criterion for a minimal central action had difficulty in focusing, which lasted in one case for 24 h.

Klapper et al. (162) re-examined one subject who had received benzetimide at some unstated time in the past. No evidence of long-term effects was found.

MEPIPERPHENIDOL

White et al. (152) gave six volunteers intramuscular injections of 100-300 mg of mepiperphenidol (1.17-3.63 mg/kg) and found that all six had blurred vision and receding of the near point of vision. These effects had nearly disappeared by the following morning. Urinary urgency and an increase of 10.8 mm Hg in mean diastolic pressure were particularly striking effects. The mean systolic pressure increased by 5.9 mm Hg, and the mean heart rate by 45.3 beats/min. The mean breathing rate increased slightly. All the subjects complained of dryness of the mouth, and all but one of becoming drowsy and of feeling unsteady in walking. The dose of mepiperphenidol

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer

increased the effectiveness of photic driving of the EEG in half the subjects and reduced the alerting reaction in one of the six subjects. Two subjects complained of eye irritation.

Nine subjects given intramuscular injections of 1.95–6.5 mg of atropine sulfate (0.031–0.064 mg/kg) and 150–300 mg of mepiperphenidol (2.21–3.99 mg/kg) all had blurring of vision and marked receding of the near point of vision, which was still somewhat farther away than normal on the following morning (mean, 10.1 in. vs. 5.3 in.). The mean diastolic and systolic blood pressures increased by 10.0 and 5.6 mm Hg, respectively. The mean heart rate increased by 37.9 beats/min. All nine subjects complained of dryness of the mouth, and eight of drowsiness and ataxia. Four of these subjects experienced slight nausea. Three complained of eye irritation.

According to the data supplied by the Sharp and Dohme Institute for Medical Research, mepiperphenidol is one-tenth as active as atropine in antagonizing the actions of methacholine chloride on the dog. If this value is used to calculate an atropine-equivalent dose for the subjects who received both atropine and mepiperphenidol, those who received sufficient doses of the two substances to yield atropine-equivalent doses of more than 0.320 mg/kg had a four-in-seven chance of developing nausea. The four persons who experienced nausea in this group were three men and one woman; the remainder of these subjects given atropine-equivalent doses above the threshold dose were two men and one women. In the group given atropine alone, the man who was the only subject who complained of nausea had received atropine sulfate at 0.037 mg/kg. Eight other subjects (six men and two women) had been given larger doses of atropine; two of these subjects complained of headache (accompanied by dysmetria in one), and a third complained of vertigo. In the group given mepiperphenidol alone, a woman who had received an atropine-equivalent dose of 0.131 mg/kg complained of slight nausea. Four subjects in this group (three men and one woman) received larger doses of mepiperphenidol than the nauseated subject, without making any complaints.

On comparing the changes in the mean values for the objectively measured properties induced by the three drug regimens used by White et al. (152), those for the subjects given both atropine and mepiperphenidol were below those for the subjects given either compound alone for systolic and diastolic blood pressures and were above those for the subjects given either compound alone for the rate of respiration and the near point of vision. The change in the mean pupil diameter for the subjects given both compounds was the same as that due to mepiperphenidol alone and greater than that due to atropine alone, whereas the change in mean heart rate for the subjects given both agents was almost precisely halfway between those due to the individual compounds. On the basis of this analysis, atropine and mepiperphenidol appear to be mutually antagonistic in relation to the regulation of blood pressure, to be somewhat antagonistic in their actions on heart rate, to be slightly mutually antagonistic with respect to breathing

Create PDF with GO2PDF for free, if you wish to remove this line, click here to buy Virtual PDF Printer